

nature

20 October 1977

Protection for the non-academic student

MOST people's image of the educational system is of a pyramid structure, with a broad base up to the age of about 16 and then a progressively narrowing superstructure as more and more students peel off into employment, until the giddy and narrow heights of the doctorate are reached. In a recent report the *Organisation for Economic Cooperation and Development (OECD)* asks some profound questions about the suitability of this structure for the present day (*Selection and Certification in Education and Employment*, OECD, 2 rue André-Pascal, Paris). One of the most interesting questions concerns the so called terminal/transfer dilemma, and has a particular relevance in a period when secondary schools are being required to accept children of a very wide range of abilities into so-called comprehensive education.

The OECD points out that such schools have to combine two functions previously fulfilled on separate tracks: that of preparing students to leave the educational system for employment, and that of training and most often selecting students for transfer to the next higher level. In practically all cases, claims the OECD, the result of this uneasy merger is that curricula and assessment practices are geared primarily to meet the needs of those who will move up, even if this number is actually a minority. The higher levels of the pyramid exert a strong influence on those beneath. It might be added that in many universities the same pattern exists for the sciences: the need to select and

train the postgraduate unduly influences the undergraduate curriculum.

At the secondary level of education the move towards comprehensive schools has clearly been seen by its proponents as a move towards greater equality of opportunity and freedom to find one's own level. If however, comprehensive schools develop in the way that the OECD describes, they will only tend to emphasise failure. Those for whom such a school was never other than their last base before getting a job will find themselves poor relations alongside those with obvious university aspirations who are catered for by examinations and school curricula with the word 'university' written all over them.

If the so-called holistic structure of an educational system acts to the disadvantage of those with 'mere' manual and technical skills, does the alternative 'aggregative' model, with tertiary education pursued in a much more diverse way, work any better? Certainly it could help to break a narrow academic grip on schools, but maybe at the high cost of fragmented tertiary courses, much poorer staff-student relationships and a general lack of direction in many students' development.

Is there a middle path, that maintains the best of academic traditions without casting out the non-academic student into outer darkness? We must find an answer in the next few years; the OECD report could be a valuable basis for discussion. □

Read all about something like it

"MANY killed in earthquake . . . where's the pun in that?" a sub-editor on *The Guardian* is supposed, in newspaper mythology, to have said whilst appraising headlines. 'Buy now while stocks last' ran the headline to a leader in *The Guardian* last Saturday; true to form the content of the leader was not about declining resources or exceptional bargains—it was another little poke at the quaint language that specialists use. Only a few weeks ago *The Guardian* was pointing out the intelligibility of *New Society* as opposed to the opacity of *New Scientist* and *Nature*. Now it returns to the fray to renounce its faith even in the accessibility of the social sciences on the basis of an advertisement in *New Society* for a book *An Eclectic Approach to Primal Integration*, and it snips a highly technical sentence on adenylate cyclase out of last week's *New Scientist* to reassure readers that we scientists are still at it.

No doubt our colleagues on the *New Scientist* know how to defend themselves against these outrages, but for our part we simply say that at least our customers know what they are getting. Anyone who picks up an article

entitled 'Analgesic activity of lipotropic C fragment depends on carboxyl terminal tetrapeptide' can be under no illusions about what they are in for, whereas readers of *The Guardian* may have to search for a serious article on Indian corruption under a heading such as 'Currying Favour'. Nor is ambiguity restricted to *The Guardian*. Every evening as we walk wearily from office to station we are assailed by placards for the evening newspapers proclaiming 'London-bound train crashes', only to discover that a freight train from Glasgow has run off the rails outside Preston.

Our enthusiasm for this theme of misrepresentation having been raised, we were just about to go on in the same vein to wonder how many horticulturalists had bought John Betjeman's *A Few Late Chrysanthemums* by mistake, how many glaciologists Margaret Drabble's *The Ice Age* and so on when an uneasy feeling crept over us. After all in the office we do get a steady stream of offers of wildlife pictures. And we get the occasional phone call asking if we have the address of the World Nudist Federation. □



Of time and the energy wars

In another of an occasional series, Alvin M. Weinberg, Director of the Institute for Energy Analysis at Oak Ridge, Tennessee, comments on a difficult trade-off

QUANTUM mechanics tells us that energy and time are conjugate variables. I am therefore much bemused these days by an observation pointed out to me by a young Swiss physicist, Daniel Spreng, that in a certain social sense energy and time are conjugates. What Dr Spreng means by this is simply that man expends energy in order to save time; moreover, when given the choice, he usually chooses to save time over saving energy. (To be more accurate, it is availability rather than energy that is usually meant in these contexts).

This surely was the case during man's early civilisation. Domestic animals did the job quicker, and slaves were acquired because they were a source of intelligent energy. The slave-owner therefore had more freedom of choice in using time than did the peasant who had to work his field himself. And each technological achievement (at least until the advent of information technology) represented such a trade-off: the industrial revolution, the transportation revolution, the agricultural revolution—always it was man in a hurry ready to spend more energy in order to save time.

Actually this exchange of energy for time has a basis in thermodynamics, despite the well-known fact that thermodynamics never treats time explicitly. In thermodynamics we learn that the minimum free energy is expended when a process, like desalting, is performed reversibly. A reversible process, however, is usually considered to require an infinite amount of time; if we wish to save time, we must expend more energy since the process will then be performed irreversibly. Thus the minimum amount of work required to desalt 1,000 gallons of seawater is 3 kWh; the actual amount used in a practical desalting device is ten or more times higher than this.

Heat transfer processes can be performed *reversibly* in a finite time if the apparatus, particularly the heat transfer surface, is sufficiently large. In that case the temperature drops approach zero, and reversibility is achieved. But we must remember that the total energy required to conduct a process is actually the sum of two energies: the usual energy which is transferred from one body to another; and the energy that went into constructing the apparatus. Ordinarily we ignore the latter. But if the apparatus becomes very large we ought not to ignore this since the energy that went into making the apparatus—the mining and refining of the metal, its fabrication, its transport—becomes very large. Thus the total energy goes through a minimum as the degree of irreversibility diminishes. To perform a task in a finite time requires more energy than to perform it in a longer time, either because we do it irreversibly or because to do it reversibly requires a very large apparatus that itself embodies a great deal of energy.

Though I am bemused by the fact that time and energy can be regarded as conjugates, I do not put this forth as a magical talisman for guiding our course in the great energy debate, but simply as an antidote for the view that thermodynamics can guide energy policy better than can the evolving values of our society, particularly as embodied in economics. This view seems to be promulgated, especially in the United States, by Barry Commoner and Amory Lovins, to mention the most notable. They assert, with truth, that in principle we can save energy if we do a better job of matching the quality of the heat source with the quality of the heat required to achieve a given end. If water is wanted at 70 °C, why use a flame with a temperature of 1,000 °C as the heat source? And in two widely read

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Camera Press

Time savers, or energy wasters?

articles, Commoner's "The Poverty of Power", which appeared in the *New Yorker* and then as a book, and Lovins' "Energy Strategy: The Road Not Taken?" which appeared in, of all places, *Foreign Affairs*, much was made of maximising this 'second law efficiency'. Indeed, the lesson thermodynamics teaches us for energy policy, according to Commoner and to Lovins, is mainly that we must maximise second law efficiency: in so doing we will save energy and create the good, that is, decentralised society, not to say eliminate nuclear energy.

But all this is so one dimensional. To be sure energy is a finite good, and we suffer from a shortage of it. But there are other goods that are finite, and of these I would consider time to be the most fundamental. This, I suppose, is one of the prices we pay for our knowledge of our mortality: we value time because we know it is limited, and we will do much to save it and to expand our choices in using it. If the underlying trade-off is between energy and time, then we mislead if we try to use thermodynamics as a primary guide to energy policy since thermodynamics gives no value to time: it is prepared to speak of processes that are infinitely slow, that ignore man's mortality.

There are those who argue that we have passed the point where energy can be exchanged for time. For example, S. Linder in *The Harried Leisure Classes* points out that our energy-intensive technologies themselves rob us of time: to maintain our gadgets, to commute, to listen to the semantic drivel that our new technologies impose upon us. And I cannot deny the merit of Linder's thesis: the Leisure Class is harried.

Yet who is to judge whether the trade-off between energy and time is to favour energy or time—the energy revolutionaries such as Commoner and Lovins, or the rest of us? We are short of energy, but we are also short of other things, like time. A free society allows each of us to make the choice, to allocate as we each decide. Economics, in the broadest sense, integrates all these trade-offs and judgments—it allows us to weigh time against energy, or for that matter, any other good against energy. I would hope the energy revolutionaries could be persuaded to be a little less revolutionary and not reject these homely truisms of engineering economics for a world they would impose on us with their emphasis on thermodynamic analysis. □

Planning Antarctica's future

The thirteen signatories to the Antarctic Treaty met in London earlier this month. **Paul Cheeseright** reports

IN THE face of increasing international economic pressures, the Antarctic Treaty is on the verge of substantial change, which could dilute, at least to some extent, the environmental stringencies practised to date by the signatory powers. The essential problem is how to control an area without having the recognised forms of sovereign jurisdiction.

For three weeks in London the thirteen consultative powers, as the signatories like to call themselves, tried to come to terms with the problem of finding regimes for the control of marine and mineral resources, while at the same time preserving the non-political approach which has hitherto dominated their scientific work. Inevitably their success has been only partial.

This inevitability is the direct result of the framework in which the Treaty powers had to work. There are thirteen of them: Argentina, Australia, Belgium, Chile, France, Japan, New Zealand, Norway, Poland, South Africa, Britain, the USA and the USSR. They make an oddly variegated bunch of states, having either geographical, historical, political or scientific links with Antarctica.

The Treaty which binds them was signed in 1958 and came into effect in 1961. Its effect has been to make the most inhospitable continent in the world a gigantic laboratory, where the work of one was subject to the inspection of all in a demilitarised nuclear-free zone reserved for peaceful purposes. "The question of man's impact on the Antarctic environment" is, the Treaty powers say, "a subject which subsumes all the main preoccupations of the Antarctic Treaty powers with regard to the area".

The early years of the Treaty's existence provoked no problems. The area was out of the way. The scientific work was expensive and only the powers involved were interested in making provision for it. But in 1969 questions were being asked in the New Zealand parliament about the mineral resources of the region. The seeds of difficulties were being sown.

There were two reasons for this. The first was that the Treaty itself made no mention of resources in the area and what to do about them. The second was that the Treaty effectively froze all territorial claims to the area for thirty years. It was at least partly in order to lay the claims to rest for a generation that the Treaty was signed

in the first place.

Seven Treaty signatories have claims: Argentina, Australia, Chile, France, New Zealand, Norway and Britain. But these claims are not recognised by the other signatories and are not accepted even among the claimant powers, because the claims of Argentina, Chile and Britain overlap. In addition some 15% of the Antarctic land mass is unclaimed. The danger of the mineral resources problem was that it could bring out into the open the territorial claims, because in the event of mineral exploration or exploitation there would need to be some jurisdiction over the activity.

Rights questioned

In recent years however, the rights of the Antarctic Treaty powers to define even loose methods of control of the area have been questioned. The United Nations Law of the Sea Conference embraced the concept that the oceans were the heritage of mankind. There is no reason, some Third World powers have argued, why the same concept should not be applied to Antarctica.

In the event the problem of mineral resources has not had quite the same urgency as marine living resources. The latter involve primarily the krill, a crustacean seen by some scientists as a major untapped source of protein which could be harvested in sufficient quantities to double the world's annual fishing catch.

The depletion of the region's whale stocks has led to an increase in krill stocks, pointing to the role of the

creature in the ecosystem of the southern oceans. But detailed knowledge of the ecological interaction of the krill and other Antarctic marine species has not yet been accumulated. It is not even known whether the Antarctic contains one or numerous distinct krill populations.

Nevertheless the proliferation of the krill and its ready exploitation have already attracted the attention of West Germany, which has reported catches of 40 tonnes an hour, the USSR, Japan, Chile and Poland. It has also been the subject of survey by the Food and Agricultural Organisation (FAO).

The implications of all this for the Antarctic Treaty powers at the London meeting were serious. If environmental damage was to be prevented then it was necessary to find a regime of conservation, but the regime would have to be voluntary. Unless national territorial claims with 200 mile economic zones were to be recognised in defiance of the Treaty and of some of its signatories, there could be no policing. At the same time the lack of any internationally accepted system of authority meant that any nation could move its fishing vessels into the area.

The Treaty powers are planning a conservation regime, which they hope will be ready for signature at the end of next year. Such a regime will embrace several principles, they decided. In the first place it will include the "rational use" of resources, so harvesting is not prohibited. In the second place they are mainly concerned with the seas south of latitude 60°S.

The third principle is that the regime would exclude catch allocations. At the same time, however, it could involve a total catch figure. It is not clear how it is possible to have the latter without involving the former.

The fourth principle is extension of the freezing of territorial claims into the conservation regime. The effect of

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this is to ensure the rights of free access into the southern waters adjacent to the continental landmass. While this constitutes a leap over a major political hurdle and ensures a degree of order in the affairs of the continent, concomitantly it means looser control.

But the way has been opened for widening the international acceptance of the Treaty. The powers are considering inviting countries like West Germany to take part in the drawing up of a conservation regime, and international organisations like FAO could send observers.

In general the Treaty powers are anxious that any nation which is active in the area should accede, so that practical answers may be provided to practical questions. The Treaty is not closed, but the powers remain concerned about the possibilities of hegemony by an international organisation which might diffuse authority still further and act not as a spur to action but as a block.

Interim guidelines

In the meantime, pending a definitive conservation regime for marine living resources, interim guidelines have been established. But they do not go beyond commitments to cooperate in research, coordinate shipping programmes and the exchange of catch statistics. The

guidelines are accompanied by an acceptance of the need not to harvest the resources to the extent that there will be depletion.

In common with the principle adopted for marine living resources, control of the continent's mineral resources will not affect the position on territorial claims. But the Treaty powers have made their strongest commitment so far not to engage in mineral resource exploration until a regime of control has been established. This they intend to work towards. That said, however, there is no theoretical reason why a company should not start drilling in the area.

But this is not likely. Mining companies have already pointed to the distance of Antarctica from the world markets, the easier access available to resources in more hospitable climates and the expense of coming to terms with land covered by an ice sheet. The reservations of the companies are also held by experts from the Treaty powers. Their discussions and conclusions have taken a good deal of the pressure away from the need to reach quick solutions on control.

It is thought that it will take at least five years to amass the scientific and technical data necessary to provide a foundation for exploration and subsequent exploitation if conditions are to

be found which safeguard the continental environment. Exploratory drilling itself might take ten years. In short the possibility of exploiting mineral resources is at least 15 years away, and probably more like 25.

This assumes that the continent has hydrocarbon and other mineral resources economically worth exploiting. This is not proven. Geological history indicates the possibility, but so far only traces of mineralisation have been found on land, although unmetamorphosed tertiary sediments, often associated with oil and gas, have been discovered in areas offshore.

There is no technology available for oil production all the year round in Antarctica. The concepts for self-contained installations on the seabed exist but that is all. On land there is no technology available for drilling through the ice shelf.

Meanwhile the Treaty powers have the immediate problem of ensuring that the information they collect is made available to all who need it. The continent is vital within the international meteorological system, but the powers have discovered that only 25% of their observations ever reach the world system. Somewhere between the Antarctic research stations, the global telecommunications system and the world weather watch, the rest evaporates. □

Ninety days and more

As the Windscale inquiry draws to an end, **Eben Wilson** sums up progress

THE Windscale inquiry has become an institution—the British democratic tradition gone wild in the interest of a public debate about nuclear power. In its ninety days, independent and government scientists from throughout Britain and abroad have for the first time been disagreeing and airing their uncertainties in public, while wrestling with the thorny problem of the close links between nuclear power development as an energy option and its social and political consequences for the future.

The inquiry has taken over a civic hall of 1960s' architectural utility in Whitehaven, a small town perched almost inaccessibly on the Irish Sea coast to the West of England's Lake District, eleven miles from Windscale. Inside one large room, the inquiry inspector, Mr Justice Parker, a high court judge, and his two assessors, Sir Frank Warner and Sir Edward Pochin, sit trapped at a green baize table among a pile of papers. They look out

on five rows of lawyers, scientists and environmentalists who hide behind their green baize tables loaded with documents, articles and studies on nuclear power.

Out of that sea of paper a pile of daily transcripts now four feet high has appeared. Many say these will become an historic document, the quintessential reference work for the international nuclear debate.

Although the inquiry is specifically trying to decide on a planning application to build an oxide fuel reprocessing plant (THORP) alongside Britain's present Magnox reprocessing facilities at Windscale, it has become the platform for discussions on Britain's future energy options. Ranged against British Nuclear Fuels (BNFL), who own the present plant, is an array of pressure groups and individuals, from the established Friends of the Earth (FOE) to local housewives worried about their children.

The Friends of the Earth have waged

war on the economic case for reprocessing and have convinced the inquiry that the British taxpayer will have to pay somewhere between £300 million and £500 million to recycle spent oxide fuel from Britain's advanced gas cooled reactors (AGR). The exact figure depends on where the price of uranium lies between \$30 per pound and \$100 per pound, and a notional price for plutonium above £90,000 per tonne. BNFL estimate an ex-works price for recycled fuel of £260,000 per tonne or above depending on whether permission is given for a 1,200 tonne per year plant with capacity for foreign reprocessing contracts, or a 600 tonne a year plant only for British fuel.

The FOE alternative consists of importing uranium for fuel, storing fuel elements on a long term basis and abandoning reprocessing. They have asked for a ten-year delay to THORP while long term storage is researched. If this fails, they say, the delay would at least give time to try to evolve safeguards against plutonium proliferation.

British Nuclear Fuels have an unhappy history of Magnox fuel elements deteriorating in their cooling ponds at Windscale and have revealed that AGR fuel, still less than ten years old, is already showing signs of corrosion.

They view reprocessing as good waste management and point out that against the cost of reprocessing has to be put the cost of fuel storage, which nobody knows, and that storage itself does not guarantee that fuel elements will not eventually have to be dealt with either by glassification (at £450,000 per tonne), or even reprocessing to prepare fuel for glassification.

Scientific struggles

The real scientific struggle has been over the level of discharges from Windscale into the Irish Sea. These contain long-lived α -emitters, ^{239}Pu and ^{241}Am and shorter lived isotopes of ^{137}Cs .

Hours of evidence and reams of paper have shown disturbing inadequacies in the research done by the Ministry of Agriculture, Fisheries and Food (MAFF) and the Fisheries Radiological Laboratory (FRL) into transuranic marine pathways back to man's environment. The Isle of Man government produced Dr Vaughan Bowen, an expert in marine pollution from America, who castigated the FRL for its methods of measuring silt movements and biological pathways. Dr Brian Wyne for the Network for Nuclear Concern and a research lecturer at Lancaster University, cross-examined both MAFF and FRL at length on the critical group method of evaluating derived working limits for the consumption of contaminated organisms. Cod, muscles, porphyra (an edible seaweed), silt and more have been metaphorically and literally dissected to examine possible synergistic effects of low level contamination or the possibility of biological systems concentrating transuranics.

The result of the public analysis has been a mixture of scientific humility and confusion, and human arrogance and anger, each coming from both objectors and government scientists. Researchers using the transcripts after the inquiry will find long lists of documents showing the present confusion over the effects of low level radiation and the concept of a threshold for radiation below which humans are not affected.

Much evidence has been presented attacking the present International Commission on Radiological Protection (ICRP) limits. Dr Sadao Ichikawa from the Laboratory of Genetics at Kyoto University, Japan, in particular, presented disturbing evidence on the long term genetic effects of low level radiation.

The weight of all the technical evidence threatened to swamp the inquiry in its sixteenth week in a shower of facts when Justice Parker admitted that he and his assessors were falling behind under a deluge of documents.



Awaiting assessment: the Windscale plant

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Making assessment: Warner, Pochin, Parker

Equally, however, it has given the inquiry a certain authority, so that when Thomas B. Stoel from the National Resources Defense Council (NRDC) in Washington, DC, came to suggest that a full environmental impact study should be done for Windscale, Justice Parker was able to wonder aloud and a little wearily what he had been doing for the past 74 days.

The overall impression of the discharge arguments has not been that BNFL are pumping death every day into the sea, but rather that nobody yet really knows what happens to the radiopathogens once they get into the silt and marine food chains. Both Dr Wynne and Dr Bowen have pleaded that independent bodies be funded to allow a plurality of research into radiation hazards.

Justice Parker could be forgiven for sighing and wondering what all this has to do with building a reprocessing plant now. He is known to favour risk comparisons with other industries and a practical approach to hazards. He has acted swiftly on the more alarmist claims made at the inquiry. The Lake District's water has been tested for tritium, air sampling is being done at Ravenglass a few miles from Windscale, and twenty local volunteers are

undergoing whole-body monitoring tests at the plant. The scientific groups on both sides of this application have agreed that the tests will prove nothing—an ironic consensus, as witnesses have usually retreated behind the armour plating of “not enough information” when under pressure.

The Oxford Political Ecology Research Group (PERG) faced the problem of getting enough information when they attempted to calculate the consequences of an accident at Windscale. A computer programme was provided by the Safety and Reliability Directorate, but agreement could not be reached on a possible mechanism for an accident that would provide the inputs to the programme.

Stunning realism

Justice Parker has gained a reputation for realism. He stunned the objecting groups at the beginning of July by declaring that alternative energy strategies in place of nuclear power would have to be presented showing “how and when and to what extent, and at what cost the energy gap can be filled”.

Strangely perhaps, the presentation of alternative energy sources has been made by ex-motorway campaigner, John Tyme. Stephen Salter of Edin-

burgh University explained wave-power systems, prophesying that the first string of 50 "nodding ducks" will be in the sea off the north of Scotland by 1984. Dr Peter Musgrove of Reading University put four hundred windmills into the Wash in the 1990s to produce around a quarter of Britain's energy demand at a price not far above the cost of coal now. His figures are becoming firmer every year. Solar power, biomass, seabed organic sediment fluidisation, combined heat and power, fluidised bed combustion were all at different stages into the future with corresponding scarcity of operating costings. But more traditional benign systems like water power, tidal power, and of course conservation were available options today.

The problem is money, either for research or first generation operating plants. All the witnesses giving evidence on soft energy paths claimed Britain was being slow in investigating alternative options. Amory Lovins' book *Soft Energy Paths*, which appeared during the inquiry, rapidly became the 'bible' on the way and the will for a move towards these options.

Time-scale has been Justice Parker's concern. John Tyme's trump card has been Yorkshire miners' leader Arthur Scargill, presenting the case for bridging the gap before a soft energy future with a huge expansion of the coal industry. This almost worked, although BNFL did a pretty savage job in cross-examination on both the productivity record and safety record of the coal industry in comparison with the nuclear industry.

The energy establishment represented by the Central Electricity Generating Board (CEGB), the South of Scotland Electricity Board (SSEB) and the Department of Energy itself faced a barrage of criticism from objectors. Surplus generating capacity, the downturn in energy demand since the oil crisis, and a radical change in

energy coefficients of new innovations were cited as proof that present energy demand forecasts are far too high. In particular, Dr Peter Chapman from the Open University Energy Research Group tied up the present view of energy relying on supply needs, with the need to change to a soft energy scenario based on energy income.

The nuclear industry is of course cursed with one emotive argument above all—the Bomb. The inquiry has not denied that nuclear warfare is a terrifying prospect. But British Nuclear Fuels has been at pains to deny that it is a conceivable prospect for future generations living with large quantities of plutonium resulting from reprocessing. Britain will be custodian to fifty tons of plutonium by the end of the century if THORP goes ahead. Five tons would fuel the first commercial fast reactor CFR 1. Throughout the inquiry the shadow of safeguards and security has been cast over the proceedings.

The fall-out from this has gone in three directions—towards terrorism, international nuclear diplomacy and civil liberties. BNFL thought they had squashed terrorism by their insistence that "measures" could be taken to prevent theft of plutonium. "Spiking" (making it radioactive), only returning it on contract as refabricated fuel, and the sheer difficulty of making a device that explodes rather than produces a large criticality excursion, are enough of a deterrent to BNFL. They add that there are easier ways to achieve political ends without dabbling in radioactive materials.

But BNFL's confidence was rudely shaken by two American witnesses. Professor Arnold Wohlstetter from the University of Chicago has explained to the inquiry that a nuclear device has been exploded using reactor-grade plutonium in the USA. Tom Cochran, again from the NRDC in Washington, DC, presented evidence outlining the

numbers needed for an armed raid on a nuclear plant (around nine outside and three inside.)

Avoiding proliferation

FOE have maintained a consistent line on the international threat of plutonium proliferation. They see as inevitable the political pressures for reprocessing contracts that specify return of plutonium after a certain period. The motivating force of Walt Patterson, their scientific adviser, has been proliferation, with a specific image of a world where shipments of plutonium will become familiar, leaving contempt for safeguards to provide the inevitable disaster.

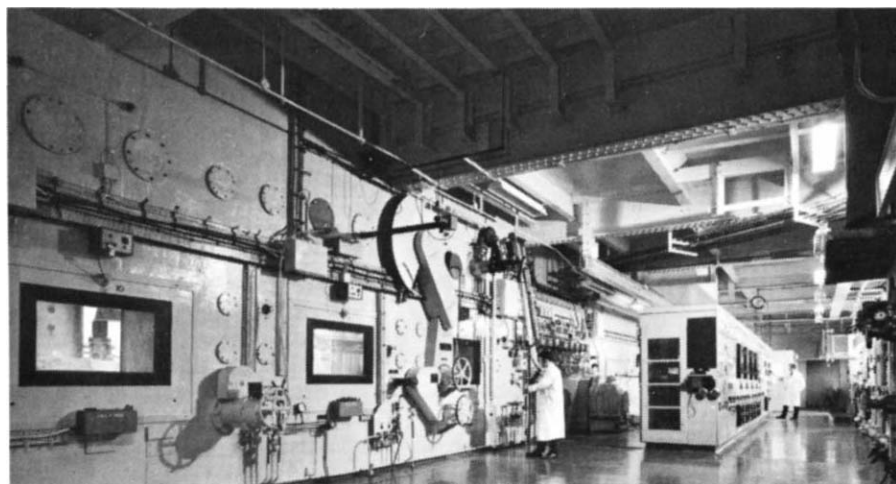
Positive suggestions to avoid proliferation have been made by Professor Joseph Rotblat from London University. He favours an international bank for buying in, storing, and allocating nuclear materials to establish the nuclear industry as a global enterprise. Research into storage options, reprocessing and the fast breeder would all be done under international licence. Reprocessing would be abandoned until proved essential.

Whatever the solution to proliferation, the attempt is to avoid the Orwellian 'plutonium economy'. Both the National Council for Civil Liberties and Justice made pleas that future generations would not be committed to a life of surveillance, power parks guarded by automatic weapon carrying police, and hunts for subversives.

By holding a mental map of all this in his head, Justice Parker has gained immense respect for the intellectual feat he is attempting. The weight of evidence is with the objectors, there being more of them, but he will be typically concerned with common sense applied to reprocessing now in Britain.

In retrospect Whitehaven has been an essential part of the spectacle. Inquiry faithfuls staying in enclaves throughout the town have been indulging in increasingly frenetic energy outlets as time goes on. Beach barbecues, pony-trekking, and a treasure hunt, where a vital clue was missing leaving ten stranded inquirers tearing around the Cumbrian countryside in complete confusion, have added colour to the interminable days of evidence and cross-examination.

Foreign visitors comment on the lack of armed police at the doors and the politesse of the participants. Perhaps it's one of the problems of the inquiry that the British will never see something as *that* important. The feeling now as summing up begins is "we wish the end of term party would come". But although the work is ending for some, for Justice Parker it is just beginning. □



Windscale's head-end plant: no longer working, it has reprocessed oxide fuel

BNFL

PROFILE

Chris Sherwell on Professor Geoffrey Allen

SRC's new head, old hand

GEOFFREY ALLEN heads a 400-strong organisation that wields a budget of over £125 million and influences the fate of the great majority of Britain's basic researchers. He is not new to its workings. But he is new to his post. He took over from Sir Sam Edwards as chairman of the Science Research Council at the beginning of the month.

The job demands practical experience of research and of administration and, if possible, of the 'real world' outside both. It also demands more than a little political nous. An erratic economy makes consistent support for Britain's research effort hard to achieve. To fight for money on behalf of scientists at large, and then dispense it on behalf of the community at large, is just as difficult.

So how did Professor Allen come to it? Under the British system which chooses to recruit through the grapevine, he wasn't the first man approached. But he is realistic about this. He accepts the system and says he has used it himself. Equally, he'd be happy if jobs like his were advertised—provided the minister concerned (at Education and Science) was not constrained by the list of applicants. He is also sure that if the job had been advertised, he wouldn't have applied for it.

He is admirably qualified, of course: a polymer scientist of 48 elected last year to the Royal Society, he has SRC and industry (especially ICI) connections going back many years. The problem was that he had recently taken up the chair of chemical technology at Imperial College, and was starting a research group there; he couldn't easily ask for a 4-year leave of absence. When the approach came, however, the decision wasn't difficult for him personally; rather, it was simply a matter of working out the possibilities and reaching agreement with Sir Brian Flowers at Imperial and with the Department of Education and Science.

What Allen calls his pragmatic view of life and work comes, he says, from his background. He was born in the Derby mining town of Clay Cross, the son of an engine driver. A Methodist Sunday School teacher taught him self-discipline. And a donnish ex-Cambridge maths teacher at grammar school inspired his love for science. He sees nothing significant in the fact that he has since

'made good'. "The good thing about science", he says, "is that it doesn't signify where you come from. It's what you do that matters".

Having graduated in chemistry at Leeds and completed a doctorate there on the thermodynamics of solutions, he took the advice of Fred Dainton, now head of the University Grants Committee (UGC), and went to Canada in 1952 to learn about spectroscopy. He wanted an academic job when he returned two years later, but the pickings were thin. Geoffrey Gee, who had just left the Rubber Producers' Research Association to take up a chair in chemistry at Manchester, offered him a job "provided you work in my field and bring the techniques you've learned to bear on my problems". Allen stayed for twenty years.

Gee taught him three things: that it was possible to do good physical science on polymers; that good science is always the basis of good technology ("That coloured the whole of my career . . . I never had the hang-up some of my contemporaries had that there was something nasty about industry"); and that "scientists have to think more widely than just being a scientist"—a view reinforced by contact in Manchester with Brian Flowers and Sam Edwards, his SRC predecessors.

Allen acquired his experience of administration and industry during the 1960s, sitting on government and SRC committees and consulting for companies. Then, in 1969, in a development he sees as a crossroads, Allen helped set up the ICI/Manchester Unit Joint Laboratory he directed at Manchester. He insisted from the start that the laboratory be closed after five years, on the grounds that any worthwhile work could at least be run down steadily, any dynamic work would be picked up by ICI or the university, and any decision to re-start it could take account of experience. A little later he went half-time to the ICI corporate laboratory, also for five years.

Before Allen came to the SRC he declared all his consultancies, but served notice that he might wish to take up two while in office; this was agreed. "It would be nice to do some consulting as a working scientist", he says, "and I could do this without compromising my position". For the curious, he says his income is likely



Professor Geoffrey Allen

to stay roughly the same at the SRC, with a "chance there could be a drop".

As for the coming four years, he knows the problems awaiting him. Support for high energy physics and space, for example, can't go any lower. "They're at the margin", he says. After a declining curve in the overall budget, he now wants a flat one; an inclining one, he acknowledges, would be too much to expect. But it will probably have to come out of a larger Science Budget, that is, economic growth. Among the arguments at his disposal for a greater share of that budget, however, is one that must appeal to the scientists who dispose of it: that applications for money clearly deserving support are being turned down.

Allen here distinguishes a lack of money from a surfeit of ideas. But both are problems, even if the former is sad and the latter a sign of health. He also says that the 'dual support' system for university research is not working, save in specific cases—as when the UGC had some money for new projects and could be persuaded to put it into the Interactive Computing project for British universities.

Allen also knows the standards he needs to follow. He says he'll be running "an open office", like Flowers and Edwards before him. He is conscious of a need for proper and responsible dealings with pressure groups, though he doesn't name names. He likes tackling problems, "provided they're amenable to logic", and, he says, "I can be persuaded to change my mind. Administrators must be able to do that".

He hopes to leave his successor with an operation looking as good as the one Edwards has left him. And like Edwards, he hopes to return to science, his first love. He still works at it, after all. Last year he got a grant from the SRC.

BRITAIN

ICRF appointment

The Imperial Cancer Research Fund has announced a new director for its research laboratories. Chris Sherwell reports

THE Imperial Cancer Research Fund (ICRF) last week announced the appointment of Walter Bodmer, Professor of Genetics at Oxford, to succeed the present director of its research laboratories, Dr Michael Stoker. Stoker, a virologist, leaves the post he has held since 1968 a year early, in September 1979, when he is 61; he returns to academic life and research in Cambridge. Bodmer, 41, a population geneticist involved more recently in immunology and tissue typing, has held his Oxford chair since 1970.

Bodmer said this week that nothing significant should be read into the fact that a geneticist was replacing a virologist. In welcoming the appointment, however, Stoker described the appointment of a geneticist as "very timely", paralleling in its recognition of contemporary developments his own appointment as a virologist years earlier. Over the next couple of decades, he said, the cancer problem would be dissected at the molecular genetics level, specifically in the realm of mutagenesis. Bodmer was "one of the leading younger geneticists in the country", with an all-round ability as well as a specialisation in the human leucocyte antigen (HLA) system.

Though Bodmer will almost certainly be receiving a higher salary than he currently enjoys, the real attractions are the opportunities, facilities and support ICRF offers. He recognises that he will be giving up one sort of administration for another, but hopes not to be too tied down by such matters. There will be no teaching, no university committees, a curtailed administrative load outside but—most importantly—an allotment of space at the ICRF's sophisticated laboratories in Lincoln's Inn Fields, central London. This he can fill with his own appointments—perhaps up to a dozen—who will continue the research he has led at Oxford, though perhaps with a change of emphasis. He says he will establish an HLA typing lab there.

In spite of recent allegations about the way ICRF uses its money, which drew a sharp response from ICRF and subsequently a libel suit for the paper that made them, Bodmer sees the job as no more controversial than any similar one in universities and other

research institutes which have to justify their particular paths in research. That the job is important he has no doubt—"otherwise I wouldn't be moving". It combines, he says, "the best opportunities for biochemical research in the country". But if the post hadn't come along, he would not be leaving the country, though he admits the alternatives beyond Oxford are limited.

Both Bodmer and Stoker believe in the virtues of limited tenure as a means of maintaining high quality in research. The same purpose is also served, both men believe, by the presence of foreign visitors, another distinguishing feature of ICRF research. Stoker is a firm advocate of another controversial view relating to research—that of early retirement from positions of power. This belief, along with his appointment as Foreign Secretary at the Royal Society, which has dragged him from his research, has encouraged his own departure. He adds that two years is the sort of notice that has to be given for a job like this.

Thus the far-reaching changes of which the ICRF Council chairman, Sir Eric Scowen, spoke in his annual report (published at the end of March) are now coming to pass. The announcement of a new man to head the biggest single private research effort in the country is the outcome of a search conducted by a special ICRF committee over the past few months. Stoker's intention to relinquish his post was confirmed in the annual report; and Stoker's deputy, Dr Renato Dulbecco, retired last month. The post of deputy will remain vacant until Bodmer takes up his appointment and makes his own choice. In the meantime the deputy's role is handled by Dr John Cairns, who heads ICRF's lab at Mill Hill. □



Walter Bodmer

USSR

Space retrieval

THE cancellation of the *Soyuz 25/Salyut 6* link-up coincided most unfortunately with the celebration meeting held on October 11 to mark the 20th anniversary of *Sputnik 1*. This anniversary had been invested with considerable importance by the Soviet authorities.

In his anniversary address, Academician Boris N. Petrov, head of the *Interkosmos* programme, did his best to retrieve the situation. Reviewing the events of the last twenty years, he stressed especially the successes of the automatic space probes and the *lunokhody* and Venus descent craft in particular. Even the *Salyut* space stations, he noted, were designed to operate in both manned and unmanned modes, and in either could "solve important problems of science and technology". Since the (manned) *Soyuz* programme already had two major setbacks in the past three years (*Soyuz 15* in 1974 and *Soyuz 23* in 1976 both failing to achieve a link-up with the *Salyut* stations), Petrov naturally concentrated on the more successful unmanned ventures.

Ironically, however, it was not the crew but the automatic part of the *Soyuz 25* mission which was responsible for its failure. The main part of the link-up programme is effected automatically by the *Soyuz* on-board computer with possibly some orbital adjustments of the *Salyut* by ground control. This system operates to within 120 m of link-up, after which the cosmonauts take over the control for final approach. What appears to have happened in recent aborted missions is that the automatic system has over-shot such that the *Soyuz* has not had sufficient power reserves to attempt a second, manually-controlled pass.

Although some *Soyuz* craft have carried solar panels to recharge their batteries, it seems likely that on the *Soyuz 25* these were sacrificed to permit additional weight to be allotted to stores and/or experimental equipment. This would tie in with speculations that the crew were to attempt to break the current Soviet record of 63 days or the US record of 84 days in space.

It is not impossible, however, that a further attempt to link up with *Salyut 7* will be made before the 60th anniversary of the Revolution next month.

Vera Rich

IN BRIEF

Call for dementia research

IN its report *Senile and presenile dementias: a report of the MRC sub-committee*, the Medical Research Council recommends that senile dementia and the rare presenile dementias be designated areas of high research priority, although it does not recommend setting up a special MRC Unit yet. The main message of the report is clear however; an estimated 5–10% of elderly people in the UK suffer from senile dementia about which virtually nothing is known. The MRC should encourage clinical units prepared to undertake the intensive studies needed and support special workers within institutions caring for demented patients, who would organise research and liaise with laboratory workers

carrying out parallel biochemical, pharmacological and histological studies.

One of the most urgent needs is a clarification of the various types of dementia and their comparison with the normal ageing process. Little is known about the early stages of senile dementia and the report suggests that community studies of the elderly might be grafted onto surveys already in progress for other purposes.

German nuclear funds

IN the Federal German government's budget proposals for 1978, DM1.45 thousand million is ear-marked for nuclear research and technology. Allowing for a 4% inflation rate, this means an increase of 8.5% over the

1977 figure of DM1.3 thousand million. As before, financing the government nuclear research centres will be a main consideration and DM700 million is being made available for their maintenance.

The prototype 300MW fast breeder reactor under construction at Kalkar is costing DM2.86 thousand million at present rates and 90% is being borne by the state, with the Belgian and Dutch governments contributing DM719 million. The cost of this reactor has greatly increased year by year—DM200 million from 1977 to 1978 alone—and its completion is expected in 1983. The costs of the 300MW high temperature reactor being built at Schmehausen are running at DM1.75 thousand million.

AGRICULTURAL technology in the USA has evolved with a rapidity that is seldom perceived except by participants in its use. The consequences of migration from rural to urban areas are evident to most people, but few city-dwellers know much about what happens on the farm these days.

The change from muscle-power to petroleum has proceeded almost to completion, and is to all intent irreversible. The Council for Agricultural Science and Technology recently published an energy-use report prepared by 22 agricultural scientists. They point out that to produce today's US crops by 1918 technology would require 61 million horses and mules that would need almost half of the cropland now in cultivation to supply their food. Performing the necessary additional hand labour thus involved would call for the relocation of almost one-third of the total working population in the United States. The average cost of their toil, at \$26.50 per day, would be about nine hundred times as much as the cost of electrical energy to carry out the same amount of physical work. Very few people have first-hand memories of how crops were raised and harvested 50 years ago, and not many can comprehend what life would be like if horses were, once again, the main source of transportation.

Agricultural production uses only 3% of the energy consumed in the USA. Jet aircraft use more than this. More food is being produced than ever before, and with less human labour. In 1976, the US gained \$23 billion in foreign exchange from exporting agricultural products, as compared with \$34 billion spent on imports of energy for all purposes.

One-third of the energy used in agriculture is employed for producing fertilisers, and only 5% for production of pesticides, principally herbicides, insecticides and fungicides. One estimate is that 41% of food and

Energy for crops**THOMAS H. JUKES**

fibre production in 1960 would have been lost if pesticides had not been used. Biological control as a replacement for chemicals is 'in the news', but, as the energy-use report says, "Biological control of plant pests is the ideal in theory, but progress in practical implementation is slow and difficult."

On 27 September the Secretary of Agriculture criticised the use of pesticides as being wasteful of petroleum. He did not mention that their production and application consume less than 0.2% of the country's energy total. I wonder how he allocates

priorities: certainly he doesn't seem to be 'thinking agriculture'. There is much dissipation of fossil fuel for purposes of convenience and pleasure as contrasted with its use for food production. Tourism and family reunions must account for a substantial proportion of jet plane travel. The highways are clogged with passenger automobiles and recreational vehicles, and the waterways resound to the hum of motorboats. Give us the petroleum-powered luxuries of life, and we will dispense with the necessities. Maybe! If food really becomes scarce, there will be plenty of trouble.

Statistics are dry and boring compared with the actual rural scene. Huge tractors till the vast wheat country, clean of officious fence or hedge, in the Great Plains. The big fields of hybrid maize in the corn belt grow apace in the hot sun, nourished by synthetic ammonia, and protected by chemical weed-killers. Further west, green discs of the new centre-pivot irrigation systems dot the landscape. These are yet another replacement of manpower by energy. There has been recent criticism of the University of California for developing new farm machinery that does the work of human beings. This is like protesting against the tractor, the combine, the plough and other inventions that lifted the burden of hand labour.

Only 5% of the energy used on farms is derived from electricity. Optimistically speaking, solar energy could perhaps supply another 25% by the year 2,000, says the report. When it comes to agricultural production, no complete or near-complete replacement seems to be in sight for the dwindling supplies of petroleum.

correspondence

Melatonin in serum

SIR,—We have recently completed a preliminary collaborative study of radioimmunoassay methods in the determination of melatonin in serum in which the different techniques used in seven laboratories were compared. To each laboratory we sent the same material derived from calf serum with a melatonin level of less than 10 ng per l. To this serum melatonin was added in a final concentration of 200 ng per l or 0.86 nmol per l. Three ml aliquots of the calf serum from the pool were pipetted into glass ampoules. The calf serum in each ampoule was lyophilised and the ampoules were tightly sealed. The serum was dissolved in 3.00 ml of water and melatonin was assayed in 35 samples from 8 ampoules and was found to be 0.87 nmol per l $\pm$ 12.9% r.s.d. by our own radioimmunoassay.

Twenty ampoules were sent in December 1976 to each laboratory. They were requested to add 3.00 ml of water to each ampoule and to perform assays using their own method, reporting back the results in ng melatonin per l redissolved calf serum. The results of the different assays are given in Table 1, and are presented as ng per l and are also transformed to nmol per l according to the SI-system. It should be pointed out that the extraction techniques in the methods differed considerably between the different laboratories and that each laboratory used its own antiserum except for numbers 4 and 5 who used the same one.

The results indicate that melatonin can be measured accurately using different radioimmunoassay techniques. We now intend to repeat and extend the study to include human reference serum with different concentrations of melatonin. This will allow an evaluation of the linearity and parallelism of the standard curves, as well as give

further data on the sensitivity and specificity of the different assays. All laboratories wishing to participate in the extensive cross-validation study planned at the beginning of 1978 are welcome to submit their names to us.

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Technics rather than technik

SIR,—I thoroughly agree with what Fores and Rey wrote (1 September, page 2) about the need for making a clear distinction between science and what they propose to call *Technik*. However, I should like to point out that a well-established, but now little used, word already exists in the English language for the concept of the useful arts. This is *technics*, and I would urge the revival of this old English word, rather than the importation of a foreign-sounding one, to describe this area of our culture.

Lewis Mumford's use of it throughout his great book *Technics and Civilization* (1934, Routledge) bears out what Fores and Rey say about the need for a word to express this concept; for Mumford, unlike Bronowski and Mazlish, does not make the mistake of confusing science and technics. As he says, "science and technics form two independent yet related worlds: sometimes converging, sometimes drawing apart".

As Fores and Rey indicate, this distinction is as important in current discussions and controversies concerning the place of science and technics in contemporary culture and society as it is in historical studies. Much current discussion of the financing, control and effects of 'science' would be far less

woolly-minded if a clearer distinction were made between science and technics. However, the lack of a familiar word to express the idea of technics is I think only part of the problem. Equally important is the (perhaps only partly conscious) desire by many scientists and others deliberately to blur the distinction, and to claim for 'science' and scientists the credit for all the benefits resulting from technics.

This shortsighted attitude may have boosted funding and respect for science in the short run but in the longer run, as people become more critical of technology, it is in part responsible for the increasing disillusion with science and the growth of antisience movements. The anti-science critics are even more at fault for failing not only to make a distinction between science and technics, but also between these and the socio-political decisions which have led to particular technical developments that they dislike, and for which we are all responsible as citizens, whether scientists, technicians (surely a better word than *Techniker*, if its status can be raised) or layman. For a healthy society and a healthy science I believe we should support those, like Sir Andrew Huxley (29 September, page 366), who insist on making these important, but often unpopular, distinctions.

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The Messinian crisis

SIR,—Just a thought or two on "The Messinian salinity crisis . . . etc" by Adams, Benson, Kidd, Ryan and Wright (29 September, page 383).

A Crisis—a word often seen in the news, Is a term which most journalists

frequently use
With reference to sterling or else to a ban
On the import of Datsuns from far off Japan.

But vaporisation of Miocene seas
Is a natural process and nothing like these;
Evaporite bodies will oft mark the coast
Of an ancestral ocean which gave up the ghost.

Applying that hackneyed and trite little word
To a normal event is quite clearly absurd.
Else what might we see if left up to the Press—

The Messinian Horror or Shocker or
Mess?
ANON

Comparison of different radioimmunoassays for melatonin

Laboratory Number	Melatonin concentration of reference calf serum containing 200 ng per l or 0.86 nmol per l.	
	pg per ml	nmol per l
1	239 $\pm$ 35	1.03 $\pm$ 0.15
2	214 $\pm$ 11	0.92 $\pm$ 0.04
3	210 $\pm$ 41	0.90 $\pm$ 0.18
4	190 $\pm$ 28	0.82 $\pm$ 0.12
5	188 $\pm$ 10	0.81 $\pm$ 0.04
6	135	0.58
7	128 $\pm$ 15	0.55 $\pm$ 0.06
Mean s.d. of all 7	186 $\pm$ 41	0.80 $\pm$ 0.17

All values are mean $\pm$ s.d. Different laboratories have assayed different numbers of samples.

news and views

Observation of photon antibunching

from Peter Knight

VERY few measurable optical effects require field quantisation and the idea of a photon for their explanation. One notable example of this rare class is photon antibunching in intensity correlation of resonance fluorescence (*Nature* **265**, 683; 1977), predicted by Carmichael and Walls (*J. Phys.* **B9**, L43; 1976; *J. Phys.* **B9**, 1199; 1976) and now observed by Kimble, Dagenais and Mandel (*Phys. Rev. Lett.* **39**, 691; 1977). Whereas most optical coherence and correlation experiments have an adequate semi-classical interpretation, antibunching requires quantisation of the emitted fluorescence and the idea of a 'quantum jump'.

The radiation emitted from the resonantly-excited atoms is collected, split into two, and imaged onto two photomultiplier tubes whose output is electronically correlated. The intensity correlation of interest is

$$g^{(2)}(\tau) = \frac{\langle \hat{I}(t+\tau)\hat{I}(t) \rangle}{\langle \hat{I}(t+\tau) \rangle \langle \hat{I}(t) \rangle}$$

where $g^{(2)}(0) = 2$ if the radiation was chaotic (this positive correlation, or bunching is the famous Hanbury Brown Twiss result) and $g^{(2)}(0) = 1$ for coherent light. Fully quantised and semiclassical approaches are here in agreement. However there are quantum states of the electromagnetic field which do not have a classical description, but the experimental realisation of such states has proved difficult. States exhibiting negative photoelectric correlation ($g^{(2)}(0) - 1 < 0$) or 'antibunching' are much-discussed examples of an effect requiring field quantisation: it is hard to visualise a function $I(t)$ representing the classical intensity whose mean square is less than the square of its mean. Carmichael and Walls showed that the resonance fluorescence radiated by a single two-level system driven by a coherent field possesses the required statistical properties to exhibit antibunching. This can be demonstrated easily since $g^{(2)}$ may be expressed in terms

of the two-level Pauli operators representing the atomic source fields as

$$g^{(2)}(\tau) \sim \langle \sigma_+(t)\sigma_+(t+\tau)\sigma_-(t+\tau)\sigma_-(t) \rangle$$

so that $g^{(2)}(0) \sim 0$. This behaviour at $\tau \sim 0$ is a strictly quantum feature: the atom emits a photon at time t and is unable to radiate again immediately after having made a quantum jump down to the lower state.

The measurement of $g^{(2)}$ consists of two parts: detection of the first photon (ensuring the de-excitation of the source atom) and a delay while the atom is re-excited by the resonant driving field for the subsequent re-emission of a second photon. So

$$g^{(2)}(\tau) \propto p(t, \tau)$$

where $p(t, \tau)$ is the probability of finding an atom, initially in its lower state at t , re-excited at τ . Cohen-Tannoudji (*Frontiers in laser spectroscopy*; Les Houches 27; North Holland, 1977) has stressed that the antibunching effect in resonance fluorescence is a striking example of wavepacket reduction in action. We expect first a lack of coincidence counts at short times τ , and further expect the Rabi nutational oscillation of $p(t, \tau)$ to be reflected in $g^{(2)}(\tau)$ which

should exhibit periodic bunching and antibunching as the driven atom undergoes Rabi oscillations. For a sufficiently intense resonant driving field for which the Rabi frequency Ω is much greater than the spontaneous decay rate β ,

$$g^{(2)}(\tau) = 1 - \exp(-3\beta\tau/2) \cos \Omega\tau$$

If there is more than one atom in the observation region the interpretation is a little harder and since fluorescence could then be produced by two or more atoms this would increase $g^{(2)}$ and obscure the antibunching.

The experimental arrangement of Mandel and coworkers resembles that used by earlier groups to study resonance fluorescence spectra. Sodium atoms in a well-collimated atomic beam are prepared by optical pumping to ensure a two-level transition and are driven by an intensity and frequency-stabilised CW dye laser at the $3^2S_{1/2} F=2, M_F=2$ to $3^2P_{3/2} F=3, M_F=3$ transition frequency. The fluorescence emitted at right angles to both atomic and laser beams is collected, divided by a beam splitter, imaged on to two photomultiplier tubes, and the correlation evaluated under computer control. The atomic beam current is designed to allow only one or two atoms to contribute to the collected fluorescence at the same time. The experiment shows direct evidence for antibunching of photoelectric pulses. After various corrections have been made for accidental pair correlations produced by background laser scatter, their results are shown in Fig. 1, where the broken curve shows the theoretical single atom prediction normalised to the same peak for $\Omega/\beta = 4$.

The observed $g^{(2)}(0)$ is approximately 0.4 rather than the zero predicted theoretically for a single atom. Mandel *et al.* estimate $g^{(2)}(0)$ to be 0.5 if the field were produced by two radiating atoms located at random positions. At large τ the atomic flight time through the photomultiplier field of view decreases $g^{(2)}(\tau)$. The overall result is as expected and plainly exhibits both bunching, and more importantly, for the first time antibunching. $\square$

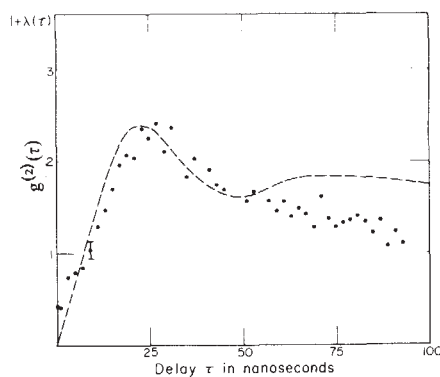


Fig. 1 Values of $g^{(2)}(\tau)$ derived from the data, demonstrating photon antibunching. The broken curve is the theoretical prediction, suitably normalised to the same peak.

Immunoglobulin genes and the immune response

by Miranda Robertson

The Tenth Harden Conference on the Molecular Genetics of the Immune Response was organised by Professor A. R. Williamson and took place at Wye College on 12–16 September, 1977.

In a meeting which was characterised throughout by lively and uninhibited discussion of almost every controversial issue in modern immunology, the outstanding topic was without question the unexpected character of immunoglobulin light chain chromosomal DNA. The surprise lies at the junction between the constant and the variable region sequences. S. Tonegawa and his associates have shown (Hozumi & Tonegawa *Proc. natn. Acad. Sci. U.S.A.* **73**, 3628; 1976) that whereas in embryonic cells the chromosomal DNA coding for the V region is on a different restriction fragment from that coding for the C region, in antibody-secreting myeloma cells they are both on the same restriction fragment. This has been widely interpreted to mean that V and C sequences are separated in the germ line, but become joined in committed B cells. But evidence from four sources, including Tonegawa's own laboratory, now shows that the V and the C sequences of myeloma chromosomal immunoglobulin DNA are separated by an untranslated 'spacer' sequence.

Discontinuous Ig gene

This explains the otherwise puzzling results of S-1 nuclease digestion of hybrids between chromosomal DNA and cDNA made on a mRNA template. S. Longacre, as reported by B. Mach (Geneva University), hybridised separate V and C region cDNAs with restriction fragments of embryonic and myeloma chromosomal DNA, expecting to find more single-stranded tails on the embryonic than the myeloma DNA. But when she compared the hybrids retrieved by hydroxyapatite extraction with those retrieved from S-1 nuclease digestion, which removes single-stranded stretches, she found that the proportions were the same for embryonic and myeloma DNA.

In a different experiment on similar principles, T. Rabbitts (MRC Cambridge) has compared the susceptibility of liver chromosomal DNA and myeloma chromosomal DNA hybridised with complete kappa chain cDNA to digestion with S-1 nuclease. Liver

DNA is assumed to be equivalent to embryonic DNA where immunoglobulin genes are concerned, so that if separate V and C sequences are joined in plasma cells the entire V-C sequence should be protected by the cDNA probe in myeloma DNA, but only separate V and C fragments in liver DNA. In fact, gel electrophoresis of the digests reveals only the C region fragments regardless of the source of the chromosomal DNA. The fate of the V region is unknown—it may have undergone partial digestion or be hidden in the C region peak—but at the very least the data imply a gap between the V and the C regions in which a short stretch of single-stranded DNA is exposed to the nuclease. Similar nuclease digestion evidence for the separation of V and C regions of active immunoglobulin genes was presented by G. Mathysens (Basel Institute for Immunology), who, however, found both V and C region fragments on electrophoresis.

The results of the nuclease digestion studies are moreover fully borne out by electron micrograph evidence from Tonegawa's own laboratory, presented at the conference by O. Bernard (Basel Institute for Immunology). Hybrids of cloned myeloma chromosomal DNA with the complementary lambda chain mRNA showed clearly in the electron microscope a single-stranded stretch of DNA which appeared as a loop between the V and C regions. Approaching the question another way, Tonegawa and his colleagues have annealed separate V and C region mRNAs with double-stranded chromosomal DNA. Because the DNA hybridises preferentially with the mRNA, this results in openings in the double strand where the RNA has hybridised with one strand of the DNA. In the case of lambda chain mRNA, two such loops (known as R loops) are separated by a roughly 1,000-nucleotide 'spacer' sequence.

It seems likely that the 'spacer' DNA has some regulatory function and that function is not peculiar to immunoglobulin genes. There are strong indications, hammered home by Mach, that spacer sequences may be a general feature of eukaryotic genes. There have recently been reports in three other systems of discontinuous genes on chromosomal DNA—in adenovirus (see *News and Views* **268**, 101; 1977), in *Drosophila* tyrosinase tRNA (Goodman *et al. Proc. natn. Acad. Sci. U.S.A.*, in the press) and mouse globin (Leder *et al. CSH Symposium*, 1977). Although the spacer sequences are not

present in mRNA, that is not necessarily to say that they are not transcribed, and the obvious speculation is that the transcript is none other than so-called heterogeneous nuclear RNA. Many have remained unconvinced that hnRNA is anything more than an artefactual aggregate of mRNA molecules, but the evidence has recently become more convincing, at least for globin (see *News and Views* **269**, 9; 1977), and indeed according to Mach, Leder finds that the size of the globin spacer corresponds to the size of the extra sequence on globin precursor mRNA. Some first steps in investigating immunoglobulin precursor mRNA were reported at the conference by R. Wall (University of California, Los Angeles) who has identified 40S and 24S precursors of kappa light chain mRNA. He has yet to find out where the V and C regions map on these precursors and whether they will hybridise with chromosomal DNA.

The answers might have been available sooner had not the early stages of the work been held up by indecision on the part of NIH about the safety of the plasmids used by Wall to clone cDNA probes for the precursor mRNAs. Delays of the same sort are affecting Rabbitts and his colleagues who are waiting for permission to clone myeloma chromosomal DNA in an *E. coli*- λ phage EKII system. Now that it is clear that cDNA is by no means necessarily a copy of chromosomal DNA and that spacer sequences may hold the answers to important questions of genetic regulation, delays of this kind are likely to lead to acute frustration, particularly where, as in the case of Rabbitts's experiments, permission has already been granted elsewhere (in Switzerland and the USA). It may not matter very much in the light of eternity whether fundamental discoveries in biology are made now or in six months' time, in Britain or in the USA—but it matters very much to biologists.

Sequence diversity in frameworks

There are no sequence data yet on the myeloma gene, but Bernard did present the first nucleotide sequence of an immunoglobulin gene: that of a cloned V region fragment of embryonic chromosomal DNA, the fruit of a collaboration between Tonegawa's group and W. Gilbert's group at Harvard. This has revealed another untranslated region, 54 nucleotides long, in the precursor sequence at the 3' end. Otherwise, the sequence corresponds largely to the amino acid sequence of

the light chain V region of myeloma MOPC 315 which produces lambda chains of subgroup ii. A curious feature of the sequence is that at three of four of the positions where it disagrees with the MOPC 315 sequence it corresponds to that of MOPC 104R, which produces lambda i light chains; in the fourth position it agrees with neither. C. Milstein (MRC Cambridge) gave voice to a general feeling that this pattern of substitutions is unlikely to be random. More sequence data should clarify the issue, but in the meantime the data are reminiscent of the amino acid sequences of mouse kappa V regions, reported by M. Weigert (Institute for Cancer Research, Philadelphia). Some residues in these chains seem to switch between closely related subgroups.

Whether more amino acid sequence data will help to clarify the question of the generation of antibody diversity is controversial. Amino acid sequences were presented at the meeting in support of an insertion theory (D. Capra, University of Texas) and of a somatic mutation theory (Weigert) but neither set of data could strictly eliminate alternative theories. It is now generally conceded that antibody diversity arises by a somatic mechanism acting on a limited number of germ-line genes (probably two or three copies for lambda chains and perhaps two or three copies for each of an unknown number of kappa chain subgroups) (Rabbitts & Milstein *Contemporary Topics in Molecular Immunology* 6, 117; 1977; Tonegawa *et al.* *Immun. Rev.* 36, 29; 1977) and there is a school of thought that the solution to the generation of diversity will be found in cellular mechanisms rather than sequences. In the meantime, the rational position on GOD seems to be agnostic.

Idiotypes and immunoregulation

Sequence data may, on the other hand, help to clarify the structural and functional significance of idiotypes, which if Jerne is right may be the basis for intercellular recognition in a regulatory network. At what levels might Jerneian immunoregulation take place? Capra presented unequivocal evidence that the so-called 'anti-Ars' idio-type on mouse anti-arsonate antibodies is determined by specific residues in the hypervariable regions which also determine antigen-binding specificity. However, there is ample serological evidence that idio-type is not always associated with the antigen-binding site, and T. Feizi for instance (MRC Clinical Research Centre, Harrow) has found that cross-reacting idiotypes on human antibodies against erythrocyte Ii antigens can also be found on antibodies which lack that binding specificity. In competitive bind-

Unification confrontation

MOST physicists hope that the electromagnetic and weak interactions are unified and this hope has been strengthened over the past few years as the Weinberg-Salam unified model of the weak interactions has notched up success upon success. But a small cloud appeared on the horizon last year; and it has not gone away.

Take the Weinberg-Salam model, add some atomic physics calculations, and the result is a prediction of small, but measurable, parity-violating effects in atoms. Preliminary results from experiments aimed at detecting these effects were reported last year (*Nature* 264, 528; *News and Views* 264, 505; 1976) and were disturbing—the predicted effects were not seen.

These experimenters now have their final results (*Phys. Rev. Lett.* 39, 795; 39, 798; 1977) and have reached a sensitivity an order of magnitude better than the prediction. No parity-violating effects are seen and the threat to the unified theories must now be regarded as serious.

There is only one foreseeable escape route—perhaps the atomic physics calculations for the bismuth atoms used in these experiments could be in error. Experiments planned with hydrogen will resolve this final uncertainty.

ing assays, the idiotypes can be identified as a heavy chain framework determinant that seems to identify a sub-subgroup.

Subgroups of subgroups were a particular feature of Weigert's partial sequence data on 22 mouse kappa light chains, and raised the question of how variable a framework residue has to be before it becomes part of a hypervariable region. (Is GOD leaky? asked Milstein). Indeed Weigert and his colleagues have now found in mouse light chains a fourth distinct hypervariable region, like that reported earlier by Capra *et al.* in heavy chains. The fourth hypervariable region (like the second, at least in those antibodies whose crystal structure is known) is remote from the antigen-binding site.

F. Melchers (Basel Institute for Immunology) was enormously interested in these results, on the grounds that the fourth hypervariable region might provide the basis for recognition in Jerne's immune network. He has indirect evidence for idiotype regulation of mouse antibodies to streptococcus A

carbohydrate, which carry the A5A idio-type. Primary immunisation with either the streptococcal antigen or antibodies against the A5A idio-type stimulates the production of idio-type-positive antibodies 40% of which bind the antigen and 60% of which do not, regardless of the priming stimulus. Melchers interprets these results—which strictly apply only to lipopolysaccharide (LPS)-sensitive cells (one third of B cells) since LPS was used to expand the number of cells *in vitro* for counting—as evidence for lymphocyte triggering by idio-type rather than by antigen-binding site in these cells.

The structural significance of idio-type arose again in connection with the T cell receptor—another crucial element in any immune network. The recent discovery that T and B cells can carry the same heavy chain idio-type and that anti-idio-type antibodies can trigger T cell help *in vitro* (see *Cold Spring Harbor Symp. quant. Biol.* 61, 1976 and *News and Views* 263, 10; 1976) has been interpreted to imply a T cell antigen receptor coded partly by genes in the pool coding for the V region of the heavy chain. However, K. Rajewsky's group, as reported by T. Cramer and T. Imanishi (Cologne University), now finds that in SJL mice T cells but not B cells carry an idio-type associated with the heteroclitic binding of the hapten nitrophenol (NP). Genetic experiments suggest that this heavy chain idio-type is expressed only in cells which produce lambda light chains and its absence in SJL B cells is due to the very low production of lambda chains by that strain. This leaves a puzzle: what produces the conformational change that gives the idio-type on SJL T cells? So far, the evidence has been against any light chain gene contribution to the 'T cell receptor.'

The proper characterisation of the T cell receptor will really be possible only when one can be isolated in large quantities. This may be soon. G. Fathman and H. Hengartner (Basel Institute of Immunology) reported a technique for producing 3-month cultures of T cells with specific reactivity in a mixed lymphocyte culture—a potential source of receptors for histocompatibility antigens.

'Hybridomas'

The final session of the meeting amounted to a progress report on the developing range of hybridomas. Since its beginning with immortal clones producing antibodies against sheep red blood cells, the hybridoma army has recruited clones producing antibodies against haptens TNP, NIP and NP; soluble proteins peroxidase, lysozyme, and chick gamma-globulin; and influenza virus surface antigens, rat

MHC antigens, T, G (A - - L) and streptococcus A carbohydrate.

To this Milstein added a line producing antibodies against an allotypic determinant on mouse molecules with the characteristics of IgD (Pearson *et al. Eur. J. Immun.*, in the press). and mouse hybrids producing antibodies against three rat bone marrow differentiation antigens (Williams *et al. Cell*, in the press). With some overlap, these antibodies seem to define the lymphoid, myeloid and erythroid populations of the bone marrow.

G. Hämmerling (Cologne University) reported the production of three hybrids secreting monoclonal antibodies against mouse H-2 antigens; these three are the survivors of five isolated from 1,000 hybridisations.

The recent extension of hybridisation techniques to T cells produced two exciting reports. Hengartner and Fathman have evidence for sensitivity to specific MHC haplotypes in two hybrids made from lymphoma cells and T cells sensitised to H-2 haplotypes in mixed lymphocyte reactions. And L. Herzenberg (Stanford

University), and colleagues has a hybrid expressing antigens coded by genes in the I-J region which is associated with T suppressor functions. The hybrid is derived from T cells in a mouse suppressed for the IgIb allotype but evidence for a suppressor factor is so far inconclusive. Supernatants from the cultured cells have shown no reliable suppressor activity against the allotype, and although sonicates have given more positive results they too are inconsistent.

Research reported at the conference represented the entire immunological continuum between what Fathman characterised as immunochemistry on the one hand and immunomagic on the other. But although structures and sequences may be cleaner than cells and sonicates, there remain problems that at this stage are not amenable to the immunochemical approach. Herzenberg was prepared to defend immunomagic. Knowing the structure of the immunoglobulin molecule, he pointed out to participants who included R. R. Porter, has not told us how B cells work. □

arranged around the inner surface of the neck near the entrance hole or ostiole. Fertile female fig wasps, *Blastophaga psenes*, enter these winter figs and lay their eggs in the neuter flowers. On completion of their development, the male offspring emerge first and bore into the flowers occupied by females and fertilise them before emergence. The males then die. Females subsequently emerge from their pupae and leave the fig through the ostiole, but in doing so become coated by pollen from the dehiscent anthers of the male flowers.

Pollen-bearing female wasps are then believed to enter figs containing a mixture of female and neuter flowers. The former have longer styles than the latter, and this prevents oviposition by the fertile females. They do succeed, however, in ovipositing within the neuter flowers, where the next generation of wasps develop, and they also inadvertently scatter their load of pollen within the fig, resulting in the pollination and subsequent fertilisation of the female flowers.

Emergence and fertilisation of the wasps takes place as before, and the fertile females now escape and invade a third type of fig which contains only neuter flowers. At this stage, obviously, there is no pollination process, but a third generation of wasps can be established within the neuter figs. Fertile females of this generation emerge in winter and lay their eggs in the neuter flowers of the winter fig (which also contains male flowers) thus completing the annual cycle.

Of most immediate interest is the provision by the plant of certain flowers, and indeed an entire inflorescence generation, which are of no

Figs for wasps

from Peter D. Moore

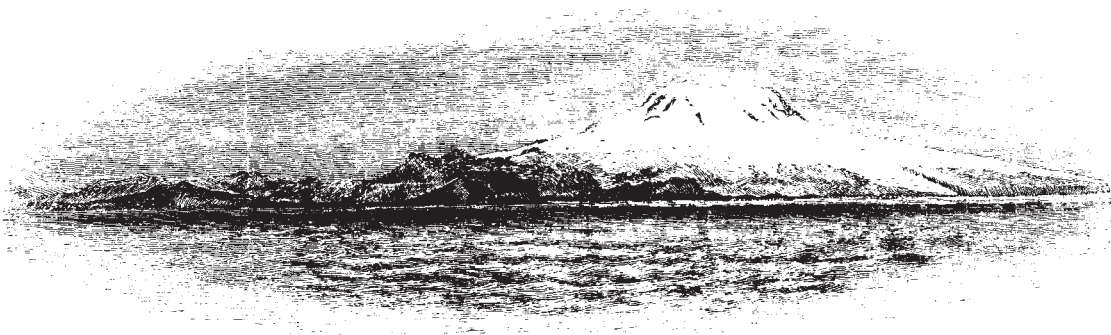
THE phenomenal success of the flowering plants may well be due, at least in part, to the way in which their floral evolutionary development has been related to the structural and behavioural modifications of insect pollina-

tors. There are on record some striking examples of complex symbiotic relationships between plants and pollinators, but few more remarkable than that of the figs (*Ficus* spp.) and the fig wasps.

The false fruits of the figs are hollow, swollen receptacles which bear on their inner surface small, unisexual flowers. In the wild progenitor of the edible fig (*Ficus carica*) a specialised winter fig is formed which contains mainly neuter flowers, with some male flowers

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A hundred years ago



THE NORWEGIAN DEEP-SEA EXPEDITION The next day was wonderfully calm, but the fog covered the higher parts of Jan Mayen. We went on shore the sea being so calm that we could step on shore without any inconvenience. The shore consisted of volcanic ash and sand, quite black, and was, higher up, covered by driftwood thickly strewn on a level surface. To the left

was a steep cliff wonderfully rich in colour, the abode of thousands of seabirds, whose inner slope, consisting of ash and scoriae, showed it to be a part of a former crater. The scientific party spread in different directions and made the best of the time in surveying, collecting specimens of plants and rocks, and drawing; one polar fox was killed. The plants found belong to very

few species, the vivid green we had seen from the ship being only a cover of moss. The flowers had just come out and all the lower part of the island up to about 2500 feet was generally free from snow, the snow lying only in the lower regions where a larger mass was gathered in ravines.

From *Nature* 16, 18 October, 526; 1877.

direct value in terms of seed production. The energy expenditure must be worthwhile in terms of maintaining a pollination vector population for the time when they are needed.

The wasps themselves have also attracted some attention recently, for the process of pollen transfer has proved more complex than was originally anticipated. It is difficult to account for pollination by a simple accidental carriage of pollen by emergent females, for as the wasp enters the ostiole of the receptor inflorescence, it is subjected to considerable mechanical stress as it forces its way between tight ostiolar scales. Indeed the entry slits are so narrow that wings and antennal flagellae are often lost. Thus the likelihood of pollen being carried into the fig inflorescence by superficial adhesion is very low. Galil and Eisikovitch (*Tijdschr. Ent.* **112**, 1; 1969) discovered specialised pollen pouches on the ventral side of the thorax in the fig wasp *Ceratosolen arabicus*, which is the pollinator of the African *Ficus sycamorus*. In the same year Ramirez (*Science* **163**, 580; 1969) described similar pouches in New World species of fig wasps. Subsequently Galil and Snitzer-Pasternak (*New Phytol.* **69**, 775; 1970) described the behaviour of the fig wasp *Blastophaga quadraticeps* as it loads and unloads pollen from its pollen pockets during pollen transfer in *Ficus religiosa*. Cleaning movements of the legs transfer pollen to the thorax and the pouches open at the touch of the legs to receive pollen. So, in many fig wasps complex morphological and behavioural adaptations aid the process of pollen transfer, which in turn ensures the fertilisation of fig ovules.

But this does not seem to work in the case of *Blastophaga psenes* and the common fig *Ficus carica*. Galil and Neeman (*New Phytol.* **79**, 163; 1977) have re-examined this relationship and have found no evidence of the complex pouches which had been noted in other fig wasps. Yet the superficial carriage of pollen was most unlikely, for not only does the wasp experience physical scouring when passing between ostiolar scales, it also cleans itself very thoroughly after emergence from the *Ficus* inflorescence and removes all external pollen.

Serial sections of wasps were taken immediately after this cleaning and the only pollen to be found was that lodged in temporary folds within the intersegmental membranes. Galil and Neeman also found that these invaginations are produced as a result of water loss from the insect after emergence from the pupa which causes infolding of the membranes. About 20–30% shrinkage of the abdomen was found to occur within the fig inflores-

cence after emergence from the pupa. They experimentally raised the relative humidity of some figs to 100% and found that wasps failed to pick up pollen.

Unloading in the receptor fig probably occurs in the reverse manner. On entering a higher humidity atmosphere the wasp's body would swell and the pollen trapped in folds would be liberated. Thus the transfer of pollen in *Ficus carica* is more complex than originally thought, but it entails neither the sophisticated anatomical modification nor the purposive behavioural mechanisms on the part of the vectors which are found in other fig/wasp relationships. Galil and Neeman point out that the common fig has about 800 stamens in a fig, compared with only about 15 in *Ficus religiosa*, the vector of which has pollen pockets. The relationship in *Ficus carica* can thus be regarded as evolutionarily primitive and energetically expensive and wasteful as far as the plant is concerned, for most of the pollen produced and disseminated is cleaned off by the unwitting vector. □

Origin of the Rio Grande rise

from Peter J. Smith

THE Rio Grande rise is a large, well known, aseismic structure stretching through at least 10° of both latitude and longitude to the west of the mid-Atlantic ridge in the South Atlantic Ocean. To say that it is well known, however, is to imply little more than that it is a conspicuous topographic feature which has received a certain amount of bathymetric attention and frequent mention in the geophysical literature. It is true that Thiede is about to publish a report (*Am. Assoc. Petr. Geol. Bull.*) in which he concludes from sedimentary and stratigraphic data that the rise was once a large island which lay 2–3 km above sea level some 75–85 Myr ago. But the origin of this sediment-covered and supposedly sunken island remains uncertain, largely because of lack of information on the nature of the basement.

Unfortunately the recent attempts by the Deep Sea Drilling Project to sample the igneous basement beneath the Rio Grande rise were unsuccessful (Leg 39, site 357). On the other hand, as Fodor *et al.* point out (*Earth planet. Sci. Lett.* **35**, 225; 1977), a large quantity of igneous material was dredged from the rise by the research

vessel *R. D. Conrad* in 1973. Dredge samples are obviously not as satisfactory as cores recovered from *in situ* rock; nor in the present case have the samples been dated. Nevertheless, from microscope, electron microprobe and bulk chemical studies on a selection of the 1973 dredge material, Fodor and his colleagues have been able to come to some general conclusions about the origin of the Rio Grande rise.

The material in question comprises basaltic rocks, some of which contain mafic nodules and megacrysts, and volcanic breccias composed largely of basaltic fragments. The bulk compositions enable the rocks to be classified as alkalic basalts, trachybasalts and trachyandesites. Moreover, the compositions of the pyroxene, plagioclase and interstitial alkali feldspar within the material are typical of volcanic rocks having alkalic affinities. The dredged samples are thus clearly characteristic of alkali basalt suites and bear no resemblance to oceanic ridge tholeiites. The compositional range (basalt to trachyandesite) also shows that the volcanic material of the Rio Grande rise has undergone magmatic differentiation.

Both alkalic composition and differentiation seem to be typical features of aseismic ridges. Indeed, the Rio Grande samples are very similar in many respects to those from the islands of Gough and Tristan de Cunha and, more significantly, to those from the Walvis ridge, the linear structure whose position almost mirrors that of the Rio Grande rise across the mid-Atlantic ridge. On the other hand, there are some distinct differences between the Rio Grande and Walvis rocks, which fact draws attention to the disadvantages of having to use dredge samples. It is reasonable to assume (although it cannot be proved) that the material dredged from the Rio Grande rise comes from the crystalline basement. But how typical is it of the basement? Does it or does it not represent all the main rock types present? There is no way of knowing. Thus the observed differences between the Walvis and Rio Grande rocks may (but also may not) simply reflect inadequate sampling. Moreover, the impossibility of knowing whether or not the sampling is representative makes it impossible to say that the Rio Grande basement does not also include tholeiitic material.

But these reservations apart, the best information available suggests that the Rio Grande rise consists of alkalic basaltic rocks covered with later sediment. And as alkalic basaltic rocks in oceanic provinces are usually associated with volcanic islands or seamounts, it

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seems reasonable to propose, as do Fodor and his colleagues, that the rise originated as a series of volcanic islands and/or seamounts which underwent magmatic differentiation and subsided as the South Atlantic opened. The evidence for subsidence is that the Rio Grande sediment contains shallow-water fossils incompatible with its present depth. Those same sediments also contain little or no volcanic material, suggesting that the igneous activity represented by the dredge samples must have occurred early in the South Atlantic's history.

However, the question of how the volcanic islands formed is still unsolved. They appear to be consistent with generation at a hotspot, although volcanic activity along fracture zones cannot be ruled out. The origin of the Walvis ridge is similarly uncertain.

European tree-trunks reveal post-glacial changes

from J. M. Fletcher

A symposium on Dendrochronology was held on 19 August, 1977, as part of the X INQUA Congress at Birmingham.

DENDROCHRONOLOGY, the dating of wood remains by the number and pattern of tree-rings has been extensively used to date wooden artefacts and timber structures from prehistoric to relatively modern times (see *News and Views* 268, 402; 1977). Some of the research reported at the symposium demonstrated that the type of tree-ring analysis involved is also revealing and dating major changes in palaeogeography over the past 10,000 years and calibrating radiocarbon ages with greater accuracy than hitherto.

In Europe, Bruno Huber (1899–1969) established German preeminence in this subject and this position is being maintained by the work of his pupils, in particular by that of B. Becker (University of Hohenheim, Stuttgart). From his comprehensive study of the subfossil tree trunks that lie at various depths in the sediments occupying former river channels in central Europe Becker has been able to trace and date with precision changes in fluvial activity and in climate from 9,600 BP (in

Preboreal time) to 780 AD, after which few trunks were deposited in the drainage systems studied.

The information on climatic changes has been derived from the changes in species present in the immediate post-glacial period and from the dating by dendrochronology of 2,000 oak tree-trunks in association with the variations in their average ring-widths (that is growth rates) at different times in the Holocene.

Periods at which depositions ceased simultaneously occurred in several river systems, for example the Danube, Rhine, Main and Werra. At other periods, for example 6,700 to 4,500 BC, the ring-widths and associated remains from peat indicate a slow rate of growth due to wet sites with a high ground water level.

The dates of major changes in the drainage system are pin-pointed by these results. For example, in late Atlantic times there was renewal of trunk accumulation due to high fluvial activity from 4,300 to 3,100 BC. Becker interprets his results for the Late Holocene as providing evidence not only for the activity of the rivers, but, from changes in the widths of the annual rings, for the clearance of forests by man with the production of deep alluvial soils.

A paper of particular interest to those concerned with the calibration of radiocarbon dates dealt with results at Queen's University, Belfast on large samples (175–200 g), each covering 20 years growth, of the sub-fossil oaks already dated in the same department by tree-ring analysis. After a lengthy period of critical examination of the human and other errors that can arise in radiocarbon analysis, G. Pearson has achieved an accuracy in which the uncertainty on each of many results is as little as ± 25 yr. The importance of this accuracy, which is comparable to that (about ± 20 yr), now achieved at Seattle by M. Stuiver on comparable samples of recent wood, lies in the reliability of the information for the first period to be examined systematically, namely from 3,600 to 4,600 BP. Whereas previous results from US laboratories a decade or so ago were interpreted as showing distinct 'wiggles' in that part of the calibration curve, the Belfast results virtually conclusively demonstrate their absence.

Two papers in the session dealt with conifers at the timberline in the Alps. Françoise Serre (Laboratory for Palaeobotany and Palynology, Marseilles) has discovered what may well be some of Europe's longest-lived trees. Most of the 38 larches sampled at an altitude of 2,100 m in the Maritime Alps (France) are over 1,000 years old. About 20% of their ring-width sequen-

ces have been cross dated and the 'signature' years compared with climatic variations. The research extends to about 500 km, the distance in the Alps over which ring-width features for this species are common to particular years.

For the eastern Alps, the High Tavern of Austria, H. W. Posamentier (Department of Geosciences, Rider College, New Jersey) described how narrow and wide rings of stone pine and larch at the timberline are related to records of glacier advance or retreat. For stone-pine, the correlation coefficient (r) is -0.69 for the years 1891–1969. The tree-ring record implies 10 or 11 periods of glacier advance since 1585: these include the years 1627–28 and 1815–22 when ring-widths were at their lowest values. □

Snow geese, lizards and sunbirds

from John Krebs

The 15th International Ethological Conference was held at Bielefeld, FRG on 25–31 August, 1977.

THERE are two genetically distinct colour phases of the lesser snow goose, *Anser caerulescens* breeding in Northern Canada. At the Eastern end of Hudson's Bay the large La Pérouse Bay colony contains 25–30% 'blue' and 70–75% 'white' morphs. At the conference, F. Cooke (Queens University, Ontario) described a remarkable long term study of the mating preferences of the two colour morphs. At La Pérouse Bay, most birds choose a mate of their own colour: only 16% of all pairs are mixed, compared with an expected 40% if birds had been choosing mates at random. In a series of experiments with captive geese, Cooke was able to show that the bird's preference for a mate of its own colour comes about through imprinting (learning during early life). The field observations and experiments established the following facts. Birds of mixed parentage choose mates at random with respect to colour, so that own colour has no direct effect on mate choice. In order for a clear preference to be established, a young bird has to grow up with parents and siblings of the same colour. Birds reared with parents and siblings of different colours showed no clear mating colour preferences, although they did avoid mating within

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their own foster group (an interesting parallel with learned 'incest' avoidance in human children). How do mixed pairs arise in nature? Cooke constructed a model based on the observed facts, and was able to show that about 40% of the offspring from pure broods must be mating at random in order to account for the observed level of mixed pairs. An appreciable proportion of birds become imprinted on the 'wrong' colour as a result of nest parasitism, for example a blue pair laying an egg in the nest of a white pair, but this alone is not enough to account for the high level of 'mistakes'. Cooke suggests two possible additional hypotheses: there is genetic variation in imprintability, or birds take a mate of the wrong colour when there is no alternative around. Cooke's study is the clearest demonstration of sexual imprinting in the wild, but the selective forces acting on the snow goose polymorphism are still a mystery. White, blue, and mixed pairs all have similar reproductive success, yet the frequency of morphs varies from one colony to another. Whether this reflects an historical accident, or the action of unidentified selection pressures remains to be discovered.

The phenomenon of sexual imprinting itself is hard to explain in functional terms. Why should birds have to learn their mating preferences rather than having a purely inborn response to their own species? P. P. G. Bateson (University of Cambridge) proposed the intriguing idea that sexual imprinting is primarily involved with recognition of kin. Bateson argued that animals should avoid mating with their own siblings (because of the adverse effects of inbreeding), but they should also prefer to mate with partners from the same population, whose genes will be adapted to the same environmental conditions. The simplest way to achieve this optimal level of outbreeding would be to learn the phenotypic characteristics of close kin, and choose a mate which is similar but not too similar to the parents and siblings. This prediction could well be tested experimentally.

Although the main theme of the conference was behavioural ontogeny, there were several outstanding contributions dealing with more ecological problems. J. A. Stamps (University of California at Davis) described how young lizards (*Anolis aeneus*) defend feeding territories, and in addition that the level of aggression shown to tethered conspecific intruders depends on the size ratio of resident to intruder. The lizards are most aggressive to individuals of the same size, which have the biggest overlap in feeding niche, and are hence the most severe competitors. L. L. Wolf (University of

Syracuse, New York) and F. B. Gill (University of Pennsylvania, Philadelphia) also reported on a study of feeding territories, and described how sunbirds (Nectariniidae) utilise their defended feeding area in a way which results in an individual avoiding recently depleted flowers so that they always harvest from those flowers which have been accumulating nectar for a long time.

Finally, T. H. Clutton-Brock (University of Cambridge) showed in a most elegant study how red deer stags settle disputes over the ownership of females by roaring contests, in which there is a gradual escalation of roaring rate until the loser gives up, presumably exhausted. There remains the nagging question of why a weak stag does not lurk in the background while two stronger males roar each other to exhaustion, and then step in to claim the hinds. □

Origin of cosmic rays

from a Correspondent

The 15th Biennial International Cosmic Ray Conference was held at Plovdiv, Bulgaria on 13–26 August, 1977.

SOMEWHERE in the Universe cosmic ray nuclei are produced and accelerated up to energies as high as 10^{20} eV. The bulk of cosmic ray nuclei, falling at the rate of 10^4 m⁻² min⁻¹ on the Earth's atmosphere, are known to be single protons and to have energies $\sim 10^{10}$ eV. The flux of such particles falls off steeply with energy ($\approx E^{-2}$) so that at 10^{20} eV the rate is very low indeed, 1 per 10 km² per 10 years. The propagation paths of the cosmic ray particles are stirred up by interstellar magnetic fields to such an extent that nearly all sense of source direction is lost. Thus the origin of cosmic ray particles remains a continuing topic for debate, and pervaded the conference. No definite solution was to emerge but some progress was achieved.

Experimentally the main attacks on the problem are concerned with the determination of the mass composition, the energy spectrum and the arrival directions of the cosmic ray particles. At lower energies ($\sim 10^{10}$ eV) interest still focuses on the relatively recent discovery of ultraheavy cosmic ray nuclei. New results were presented by the combined University of Bristol/

Dublin Institute for Advanced Studies group on the analysis of large balloon-borne lexan plastic and nuclear emulsion sandwich stacks flown from Sioux Falls, USA. A total exposure of 120 m² days was achieved at ~ 3.8 g cm⁻² atmospheric depth. A total of 274 tracks of nuclei with charge number $Z > 36$ were found, of which 96 had $Z > 65$. The charge distribution featured a peak in the neighbourhood of platinum and a high uranium group flux. The conclusion appears to be that these particles are produced by rapid neutron capture (r-process) presumably in supernovae or similar outbursts.

At higher energies up to 10^{17} eV and beyond the evidence with regard to the overall mass composition is very indirectly obtained and with controversy existing as to interpretation it is probably best (but not necessarily safe) to conclude that the mass composition is much the same as that found at lower energies, that is, 90% protons, 9% helium nuclei, 1% rest, at a fixed energy per nucleon.

For many years now it has been well established that the energy spectrum steepens significantly beyond 10^{15} eV although the explanation of the steepening remains in doubt. New results presented at the conference from the large Extensive Air Shower Arrays at Haverah Park (England), Yakutsk, (USSR), and Volcano Ranch (USA) consistently indicate a flattening in the spectrum again at close to 10^{19} eV. This flattening may perhaps represent a transition from dominantly galactic rays to extragalactic sources.

Any weight given to this latter conclusion must be strongly influenced by the almost complete isotropy in arrival directions of cosmic ray particles up to the highest energies. Harmonic analysis in right ascension of the data at these highest energies ($> 10^{18}$ eV) does suggest a measurable anisotropy creeping in, but the phase seems to change with energy and no obvious source direction is indicated. Some of the highest energy events clearly come from high galactic latitudes, strongly suggesting extragalactic origin.

The outstanding problem for protagonists of the extragalactic origin rests with the lack of cut-off above 10^{19} eV expected from the interaction of the high energy particles with the universal 2.7 K photons. This suggests that the particles observed at these energies come from distances within 10^8 light years.

In the opening invited talk at this conference V. I. Ginzburg (Institute for Nuclear Research, Moscow) argued forcefully that as the galactic halo had now been well established from radio astronomy, there really was

no problem in the bulk of the cosmic rays being of galactic origin. Later in a more detailed analysis of the situation V. S. Berezinsky (Moscow) maintained the Soviet line by suggesting a theory that could fit the total experimental data. According to his 'naive theory', at energies below 10^{19} eV cosmic rays are mostly protons of galactic origin. The phase of anisotropy varies with energy due to a focusing effect of the galactic magnetic fields. At higher energies an origin transition occurs with the cosmic rays reaching the Solar System largely coming from the local supercluster of galaxies with a maximum from the Virgo direction. For the moment this theory of the origin of cosmic ray particles seems to be the best compromise. □

Do populations regulate themselves?

by Mary Lindley

A symposium on Population Control by Social Behaviour was held at the Institute of Biology in London on 20–21 September, 1977. It was organised by D. M. Stoddart (King's College, London) and F. J. Ebling (University of Sheffield), and the proceedings will be published by the Institute of Biology.

TWENTY years ago V. C. Wynne-Edwards propounded a hypothesis to explain why populations of animals do not expand unchecked until stopped by the exhaustion of food resources. Many and perhaps most animals, he suggested, must regulate their population densities before this stage is reached, and they do that through social competition. His work on the red grouse (*Lagopus lagopus scoticus*), which was carried out at the University of Aberdeen and which he recalled at the symposium, proved him correct as far as that species was concerned. Each autumn young males take up territories in a contest with established males, and the many unsuccessful birds are cast out and usually die within six months. Thus population density is regulated.

One of the purposes of the symposium was to examine how much more evidence has emerged in favour of the hypothesis. Some of the eleven speakers addressed that theme more directly than others.

Reviewing 30 years of field work

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with great tits (*Parus major*) in Oxford and Holland, J. R. Krebs (University of Oxford) said that the birds' behaviour seems to affect population size during three stages of the annual cycle. First, during the spring breeding season the number of eggs laid by each bird can fluctuate considerably, with fewer produced when the population density is high than when it is low. This fluctuation does not seem to be a direct consequence of changes in the amount of food available. The immediate influence is more likely to be a behavioural mechanism. Second, the survival of young birds in the summer and early autumn is an important influence on the size of the population, and aggressive interactions are at least in part the cause, with heavier birds most likely to survive. The third period when behaviour seems to be regulating population is early spring, when birds compete for territories, and exclude those that are unsuccessful. But Krebs stressed that little is known about the environmental resources to which the regulating behaviour is geared.

Indications of similar regulatory influences on populations of the woodmouse (*Apodemus sylvaticus*) have emerged from equally long term studies carried out in the United Kingdom and elsewhere in Europe. J. R. Flowerdew (University of Cambridge) described how from winter to winter the number of woodmice within a population remains relatively stable. From summer to summer, however, there is considerable variation and the population is smaller in summer than in winter. The size of the winter population seems to be regulated by a density-dependent process initiated in summer. Social behaviour seems to be a part of that process, involving, for example, aggression towards immigrants and dispersal of juveniles. But more information is needed about the nature of the behaviour and about the influence of other factors, especially genetics, predation and starvation.

In primatology, however, positive conclusions seem even further away. Although primates might seem to be ideal mammals for the study of social behaviour and population structure, most of them live too long. A. Jolly (University of Sussex) pointed out that there has been pathetically little long-term fieldwork with primates. Hope is offered, however, by the lemurs of Madagascar which have a relatively short life cycle. Since 1963 sixteen studies have been carried out in the favourable circumstances of the Barenty Reserve, and although the objectives have been different in each case, the data can be combined to

represent a long-term study of the sifaka (*Propithecus verreauxi*) and *Lemur catta*. A picture is emerging of stable populations, with *P. verreauxi* maintaining the same territories throughout the year and from year to year, while *L. catta* fluctuates in its use of the environment. This seems to be an ideal situation for critical study of social behaviour and population regulation. And that was as far as Jolly was prepared to go.

Her fellow primatologist J. Deag (University of Edinburgh) seemed to offer even less hope. He launched an attack on primatologists, including himself, for the way they have generally discussed the adaptive significance of social behaviour. He criticised the approach of inductive reasoning, whereby hypotheses are developed to explain behaviour on the assumption that it is adaptive. This had led to much speculation, with in many cases alternative hypotheses which are difficult or impossible to test. Deag called for a more critical approach based on established facts and involving more rigorous testing of the predictions of hypotheses.

There is growing evidence that odours are involved in many inter-relationships between behaviour and population processes in mammals. Reviewing what is known so far, D. M. Stoddart (King's College, London), explained that data on the role of odours in reproduction are hard to interpret because all were obtained in the laboratory, which is very different from the field. But three aspects of population ecology provide a function for odour. First, social dominance can be marked by odour in several species, including rabbits and marmosets. The emerging picture is of a rise in social dominance associated with increased production of scent, increased marking behaviour and a change in the composition of the odorous secretions. Second, odour can be used to mark out and maintain territory. It chiefly deters intruders and serves as a landmark by which the occupier recognises its own territory. Third, odour seems to be involved in the transmission of danger and warning signals, although so far the evidence is not strong. As Stoddart pointed out, the role of odour in population regulation is another underdeveloped area of investigation.

By the end of the symposium the only conclusion to have emerged was the familiar plea that more work needs to be done. Wynne-Edwards professed himself completely content that discussion about his hypothesis is still alive. As he said, you cannot expect answers of the sort given at meetings of the British Association in Victorian times. □

review article

Recent excitement in the DNA replication problem

Bruce Alberts* & Rolf Sternglanz†

It is now possible to reproduce most of the reactions involved in DNA replication using prokaryotic enzymes in vitro. Such systems have revealed that DNA replication is a complex process depending on a relatively large number of proteins, and that nucleoside triphosphate hydrolysis energy is used at several discrete steps. Much of the complexity of DNA replication may arise from the need for extreme copying fidelity.

DNA REPLICATION represents a central biological problem whose general outlines were solved "in theory" by Watson and Crick. For the past 24 years, geneticists, biochemists and molecular biologists have been hard at work attempting to translate into chemical reality the prediction that "each chain acts as a template for the formation on to itself of a new companion chain"¹. Progress has been slower than many had expected, as both the mechanism and the enzymology have turned out to be surprisingly elaborate. The following general conclusions concerning events at a functioning replication fork now seem reasonable, and will be used as section headings in this brief review of the present status of the field. Note that we have not attempted to deal with the important problem of how replication forks are initiated, but only with how, once formed, they progress along the parental helix to duplicate the DNA.

DNA is replicated by a multienzyme complex

DNA is replicated by means of a multienzyme complex, sometimes called a "replication apparatus". This complex, because it is held together by relatively weak protein-protein interactions, has been isolated only as smaller individual protein components. Much of the challenge in this field is a consequence of the difficulty of recognising these components in cell extracts, and then purifying them and characterising their activities in larger reconstituted systems.

The first components to be identified were of course the DNA polymerases, the intricate and elegant enzymes which catalyse the actual polymerisation process, while following the dictates of the base sequence of a DNA template strand². These large enzymes (molecular weight about 10⁶) have so far failed to receive the attention they deserve from enzymologists, chemists and X-ray crystallographers. As a result, we know rather less

about their detailed chemistry than we should, despite some intriguing initial studies³.

Because it is smaller and simpler than the DNA polymerase, considerable attention has been paid in recent years to defining the chemistry and structure of a second component of the replication apparatus, henceforth designated the "helix-destabilising protein" (abbreviated as HD-protein)⁴. The prototype for proteins in this class is the T4 HD-protein (T4 gene 32 protein), originally designated a "DNA-unwinding protein"⁵, and in more recent years called by some a "DNA-melting"⁶ or "DNA-binding"⁷ protein. Quantitative data reveal that by binding tightly, co-operatively and specifically to single strands, the T4 HD-protein and its analogues in other organisms greatly reduce the thermodynamic cost of DNA helix melting within the cell^{5,6,8,9}. However, as discussed below, additional energy seems to be needed to open the parental DNA helix ahead of the fork. Further, HD-proteins interact in specific ways with many other proteins which function on DNA, and can thereby play a deciding part in determining their mode of action^{7,10-13}.

The other proteins which interact with the DNA polymerase and helix-destabilising protein to complete a replication apparatus are less well characterised. Here geneticists have contributed much to recent advances. Their work forecast the complexity of *in vitro* systems now in use, revealing as early as 1963 a requirement for numerous gene products in a large replication apparatus^{14,15}. Moreover, the many mutants they isolated made it possible for biochemists to develop "*in vitro* complementation assays". Such assays, which quantify the activity of mutationally defined proteins, have allowed numerous essential proteins of previously unknown function to be identified and purified to near homogeneity from *E. coli*^{10,11}, bacteriophage T4^{16,17} and bacteriophage T7¹⁸⁻²⁰ replication systems. The development of an *E. coli in vitro* DNA replication system using only soluble components²¹ was another major advance. So far, the number of putative replication fork proteins identified by genetics and/or biochemistry is at least 13 from *E. coli*^{10,11}, 6 from the T4 system^{17,22} and 4 from the T7 system^{18-20,23}. Possibly some of the extra proteins in the *E. coli* system are essential because the assembly of the replication complex needs to be much more carefully controlled in a bacterium than in a bacteriophage.

An interesting and so far unexplained feature of these multienzyme complexes is the large number of different replication proteins which hydrolyse nucleoside triphosphates to nucleoside diphosphates and inorganic phosphate. These include *dna* B protein²⁴, copolymerase III*²⁵, replication

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§ The new term "helix-destabilising protein", and the HD-protein abbreviation, are suggested here after consultation with other workers in this field. They are designed to avoid confusion with other types of proteins which seem to use ATP hydrolysis energy to aid in DNA-helix unwinding (see text). As an example of the convention proposed, the extensively characterised *E. coli* (3) and calf (4) analogues of the T4 HD-protein (gene 32-protein) are to be designated henceforth as the *Eco* HD-protein I and the calf HD-protein I, respectively. This nomenclature is designed to be descriptive enough to emphasise the striking homologies which exist between HD-proteins from different organisms, without focusing on any one of the possible consequences of thermodynamic DNA double-helix destabilisation (e.g., double-helix unwinding, acceleration of DNA renaturation, and single-strand extension; see ref. 5).

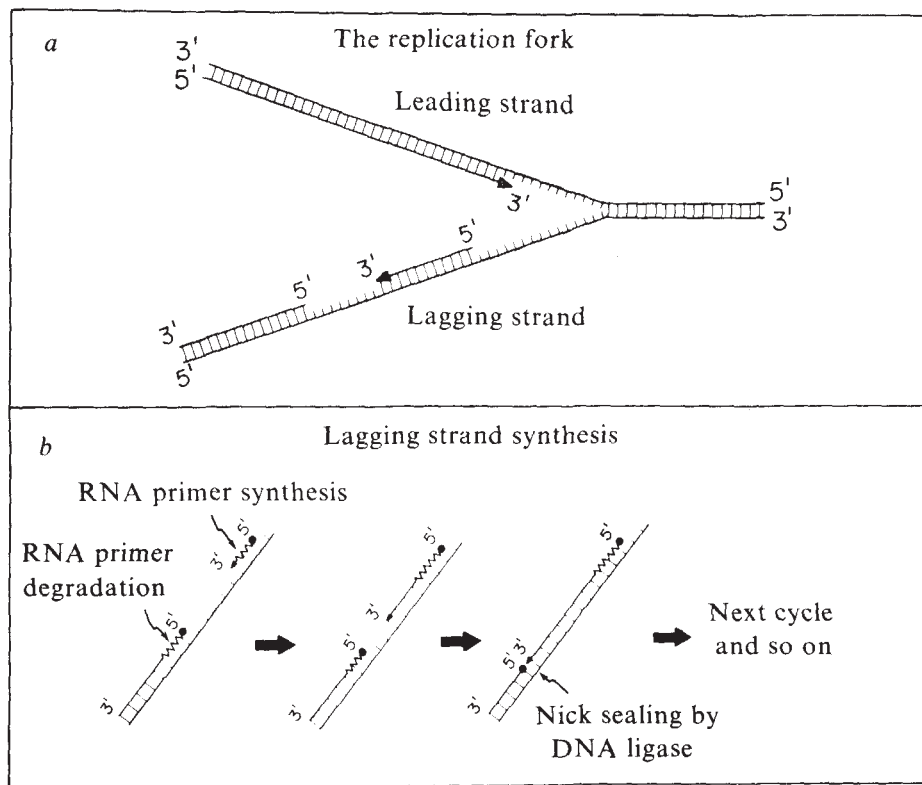


Fig. 1 *a*, Schematic representation of a replication fork, in which synthesis on the leading and lagging strand differs, but is carried out by the same type of DNA polymerase. *b*, Lagging strand synthesis in more detail: a cycle of synthesis and degradation of the RNA primer believed to start Okazaki fragments (for details, see ref. 38).

factor Y²⁶, *rep* protein²⁷, and presumably DNA gyrase²⁸ from *E. coli*; gene 44/62 protein and gene 41 protein from T4^{23,29}; and gene 4 protein from T7³⁰.

Fidelity constraints on the mechanism probably generate the observed asymmetrical replication fork

Most of those working on the biochemistry of DNA replication in the early 1960s tended to view replication as a relatively simple process. Cairns had shown by autoradiography that the two parental polynucleotide chains become separated from each other, and at about the same time become paired with newly made complementary chains, at a structure termed a "replication fork"³¹. The simplest mechanism to draw was one in which DNA polymerases continuously elongate the DNA chains on the two sides of this fork. Because of the antiparallel orientation of strands in the DNA double helix, only one of these DNA polymerases could proceed by chain elongation in the 5' to 3' chain direction (by attack of a 3'-OH of a polymer chain end on the activated 5' end of an incoming 5'-deoxyribonucleoside triphosphate monomer). A second type of DNA polymerase had to be postulated for which the growing chain end carried the 5' triphosphate activation, and the incoming 5'-triphosphate monomer was "backed in" to present its 3'-OH for the polymerisation reaction. Efforts to find such a second DNA polymerase were unsuccessful. Moreover, pulse-labelling with ³H-thymidine revealed 1,000–2,000 nucleotide-long "Okazaki fragments" of DNA at the growing fork³², apparently synthesised in the 5' to 3' chain direction only³³, and subsequently joined together by DNA ligase^{34–36}. These observations suggested that at least one of the two daughter DNA chains grows discontinuously at the replication fork, and in conjunction with detailed electron microscopic analysis of replicating molecules³⁷, led most workers in the field to switch to favour the type of model for replication schematically illustrated in Fig. 1*a*. Here, because of the asymmetry of synthesis on the leading and lagging sides of the fork, only a DNA polymerase which elongates chains in the 5' to 3' direction is required. However, fresh complications arise from the (at first sight perverse) inability of known DNA polymerases to start DNA chains *de novo*^{2,39,40}. Such new chain starts are

required at frequent intervals on the lagging side of the fork (Fig. 1*a*). Partly for this reason, a new primer-generating polymerase was sought, and eventually found in the *E. coli* replication system⁴¹. Its properties, combined with data from another system^{42,43}, made it seem likely that Okazaki fragments are initiated on short RNA primers which are elongated by DNA polymerase and then erased (and replaced by DNA) before DNA ligase-catalysed sealing of the lagging-strand DNA fragments. One such hypothetical cycle of primer synthesis and degradation is schematically illustrated in Fig. 1*b*.

The type of mechanism outlined in Fig. 1 seems at first sight much more complex and unwieldy than necessary. Is it possible that nature has been foolish and wasteful in engineering these crucial processes? In attempting to answer this question, it is important to recognise that DNA replication must be extremely accurate. Indeed, the observed templating fidelity is such that only one mistake is made per 10⁹ to 10¹⁰ base pairs replicated in *E. coli*⁴⁴.

The initial finding that DNA polymerases require a pre-existing primer chain to begin synthesis^{39,40}, began to be understood in the context of fidelity when it was found that for polymerisation, both T4 and *E. coli* DNA polymerases demand a Watson–Crick base-paired residue at the 3'-OH terminus of the primer to which nucleotides are being added. When confronted with a template-primer with a terminal mismatch, these polymerases use their built-in 3'→5' exonuclease activity to clip off unpaired primer residues by hydrolysis; this continues until enough nucleotides are removed to regenerate a base-paired terminus and create an active template-primer⁴⁵. As a result, these polymerases will efficiently remove their own polymerisation errors^{46–49}. As demonstrated by studies with mutant DNA polymerases^{48,49}, this self-correcting feature allows the enzyme to select for the proper template base-pairing of each added nucleoside triphosphate in a separate backward reaction, in addition to its strong selection for base-pairing of nucleoside triphosphates during the initial polymerisation. Theoretically, the intrinsic mistake frequency at this first step due to base mispairings can be reduced by the same factor in the proofreading step. It has been argued from chemical considerations⁵⁰ that the intrinsic mistake frequency is unlikely to be less than 10^{–5}. In this case, at least one proof-

reading step would be absolutely required to approach a tolerable mutational load ($10^{-5} \times 10^{-5} = 10^{-10}$).

Accepting this view, it becomes clear why no DNA polymerase is able to start a new polynucleotide chain *de novo*, whereas DNA-dependent RNA polymerases can do so. We postulate that the need to be self-correcting gives DNA polymerase a stringent requirement for a perfectly base-paired primer terminus; to ask this enzyme to start synthesis in the complete absence of primer, without losing any of its discrimination between base-paired and unpaired primer 3'-OH termini, seems contradictory. In contrast, the RNA polymerases need not be self-correcting, inasmuch as relatively high error rates can be tolerated during transcription.

This line of reasoning likewise suggests a possible explanation for the failure to find a second DNA polymerase which adds deoxyribonucleoside 5' triphosphates in such a way as to cause chains to grow in the 3' → 5' direction, despite the relatively simple type of replication fork mechanism that this would allow. With such a 3' → 5' polymerase the growing 5' chain end (rather than the incoming mononucleotide) carries the activated triphosphate. Thus, mistakes in polymerisation cannot be hydrolysed away without a special enzymatic system for reactivating the bare 5' chain end thus created.

Finally, this view suggests why an erasable ribonucleotide-containing primer might be preferred for priming, rather than a non-erased DNA primer which might otherwise seem more economical. The argument that a self-correcting polymerase cannot start chains *de novo* also implies its converse: that an enzyme which starts chains *de novo* cannot do a good job of self correcting. Thus, any enzyme which primes discontinuous synthesis will of necessity make a relatively inaccurate copy (say, more than one error in 10^5). Even if the amount of this copy which is retained in the final product constitutes only 1% of the total genome (say, 10 primer nucleotides per 1,000 nucleotide Okazaki fragment), the resulting increase in overall mutation rate could be substantial. Thus, it seems reasonable to suggest that the use of ribonucleotides in synthesis of primer was of great evolutionary advantage, since it automatically marked these sequences as "bad copy" to be removed.

In conclusion, it seems likely that the type of mechanism for DNA replication sketched in Fig. 1 was specially selected to ensure that the entire DNA sequence is very accurately copied (that is, copied by a self-correcting type of DNA polymerase). Additional complexities in the mechanism, including the poorly understood requirements for protein cofactors which hydrolyse nucleoside triphosphates to nucleoside diphosphates (see above), may be similarly designed to help generate fidelity in as yet unexplained ways (see, for example, refs 51 and 52).

ATP energy can be used to aid DNA helix-unwinding at the replication fork

The early suspicion that cellular energy sources might be directly used to aid in the mechanics of DNA helix-unwinding^{63,64} has been dramatically confirmed by recent data. The first direct hint that this might be the case was the discovery of *E. coli* "DNA-unwinding enzyme I"^{55,56}. This enzyme, whose function *in vivo* is still unknown, can denature DNA only when it hydrolyses ATP, like the ATP-dependent nucleases discovered earlier^{57,58}. Later, a different *E. coli* DNA unwinding enzyme was identified, and found to correspond to the *rep* gene product²⁷. Like the above ATP-dependent enzymes, the *rep* protein can be assayed as a DNA-dependent ATPase, with ADP and P_i as products. In a recent elegant series of experiments, the *rep* protein has been shown to be necessary for DNA polymerase action on a double-helical template. Specifically, ϕ X174 double-stranded DNA circles have been used for efficient synthesis of single strands of viral DNA by the combined action of: a four-protein complex called *E. coli* "DNA polymerase III holoenzyme", *rep* protein, *Eco* HD-protein, and the ϕ X-coded *cis* A protein^{27,59}. In this reaction, the *cis* A protein nicks the (–) strand of the circular ϕ X DNA at a unique site, and attaches covalently to the 5' end of the

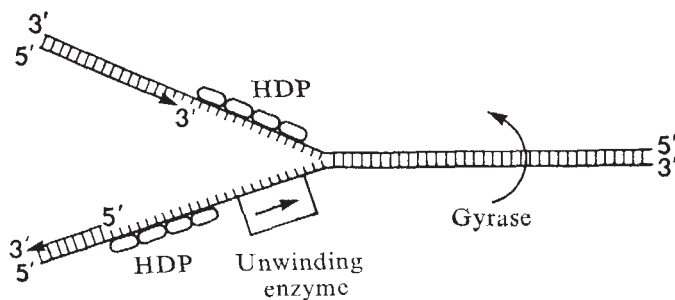


Fig. 2 Possible energetic contributions to helix unwinding. HDP stands for helix-destabilising protein (see footnote§). For a description of HD-protein, unwinding enzyme and DNA gyrase, see text. The arrows show the expected directionality of the motion generated by unwinding enzyme and by DNA gyrase, respectively, due to conformational changes occurring when bound ATP is hydrolysed by these proteins^{27,28}. The assignment of unwinding enzyme to the lagging strand side of the fork is rather arbitrary, and is based upon the fact that stoichiometric quantities of *E. coli* DNA-unwinding enzyme I seem to invade a DNA double helix by "walking" along a single strand from its 5' toward its 3' end⁶³.

DNA. The adjacent 3'-OH end created serves as a primer for the DNA polymerase, which in conjunction with HD-protein and *rep* protein synthesises the (–) strand in a modified "rolling-circle"⁶⁰ mode of replication. Approximately two molecules of ATP are hydrolysed per deoxyribonucleotide polymerised in this system.

The hydrolysis of one ATP molecule releases about 14 kcal of free energy at intracellular concentrations of ATP, ADP and P_i ⁶¹. The energy cost of melting a base pair on the template should range from about 1.2 kcal mol⁻¹ (A next to A) to 5.0 kcal mol⁻¹ (G next to G), depending on the particular base pair being melted and its nearest neighbour. (These numbers were derived for RNA helices at 25°, but values for DNA helices should be similar⁶².) Thus, coupled ATP hydrolysis can in principle make a significant contribution to parental DNA helix unwinding.

How an unwinding enzyme such as the *rep* protein may function in the replication of double-stranded DNA is schematically illustrated in Fig. 2. This scheme is designed to take into account the following facts: (1) only catalytic amounts of the *rep* protein are required for DNA synthesis²⁷; (2) the *rep* protein can hydrolyse ATP and cause DNA strand separation with *Eco* HD-protein even in the absence of DNA polymerase^{27,59}, and (3) unidirectional mechanical motion can result from coupling the hydrolysis of a bound nucleotide to allosteric changes in protein conformation (as in muscle)^{64,66}. From a recent report, the T7 gene 4 protein seems to perform a function similar to that of *rep* in the T7 DNA replication system³⁰.

Nevertheless, additional data make it clear that ATP-driven unwinding enzymes are only one of several kinds of components contributing to helix unwinding. First of all, while T7 gene 4 mutants are conditional-lethal and fail to replicate T7 DNA, all known *E. coli* *rep* mutants are viable (the rate of *E. coli* replication fork movement is slowed by only half when the *rep* gene is mutated; ref. 66). Second, an exciting new *E. coli* enzyme, DNA gyrase, has been discovered which uses ATP energy to pump negative twists into the DNA double helix²⁸, as schematically illustrated in Fig. 2. Such negative supertwisting will make the DNA helix easier to open by an estimated 1 kcal mol⁻¹ base pairs (M. Gellert, personal communication; see also ref. 67). DNA gyrase is inhibited by the drugs novobiocin⁶⁸ and nalidixic acid (M. Gellert, personal communication; N. Cozzarelli, personal communication). Since these drugs stop DNA replication immediately in *E. coli* (and many other prokaryotic systems), the contribution of DNA gyrase to helix unwinding in these system may be essential.

Finally, an important contribution to helix unwinding is

likely to be made by the helix-destabilising proteins. Apparently homologous HD-proteins have so far been found in a wide variety of organisms, including T4 and T7 bacteriophages^{5,69}, *E. coli*³, *Ustilago maydis*⁷⁰, and calf⁴. As described above, *Eco* HD-protein is required for the catalytic *rep* protein-mediated DNA melting observed with ϕ X174 DNA in the presence of the ϕ X *cis A* protein^{27,59}, although its energetic contribution to melting is not yet known. Similarly, while the T4 DNA polymerase cannot displace strands on double-helical templates by itself, the addition of intracellular concentrations of T4 HD-protein to the complete T4 *in vitro* replication system allows this enzyme to proceed through double-stranded DNA at close to the rate *in vivo* of 800 nucleotides per second²⁷. ATP hydrolysis by the T4 44/62 protein is required for this synthesis, but the stoichiometry of coupled hydrolysis is apparently less than 1 ATP hydrolysed per 5 deoxyribonucleotides polymerised (J. Barry and B. M. Alberts, unpublished results).

Since no DNA gyrase seems to be used in the T4 *in vitro* system (E. Belikoff and R. Sternglanz, unpublished results), a great deal of the 12→50 kcal mol⁻¹ required to melt one helical turn (10 base pairs) of DNA in this system may have to come from T4 HD-protein binding to the single-strands produced, as schematically illustrated in Fig. 2. Recent studies carried out on the T4 HD-protein⁶ would suggest that inside the cell, not more than 10 to 20 kcal mol⁻¹ helical turn melted should accompany its binding (assuming a concentration *in vivo* of about 10⁻⁵ M). However, stronger binding is seen when the C-terminal 8,000-dalton segment of T4 HD-protein is removed proteolytically, and the interesting suggestion has been made that the known interaction of T4 HD-protein with DNA polymerase⁷¹ and other T4 replication proteins²² similarly increases this energy of DNA binding at the replication fork⁷².

To summarise the situation as it seems to be now, a large part of the energy required for DNA helix unwinding ahead of the DNA replication fork can, in principle, be derived from the sum of the several forces illustrated in Fig. 2. No one of these proteins need be sufficient to accomplish the task alone. Surprisingly, different replication systems seem to use a different balance of the mechanisms illustrated, in some cases all three. Finally, the fact that DNA polymerase I of *E. coli* can use double-helical DNA templates in the absence of any of the proteins in Fig. 2 (ref. 2) means that considerable energy for helix unwinding can also come from the deoxyribonucleoside triphosphate hydrolysis which accompanies polymerisation. We can assume that the replicative DNA polymerases also derive energetic contributions to helix unwinding from this source.

Okazaki fragments need not all arise from *de novo* priming events

In this section we discuss the current status of work on the nascent DNA chains known as Okazaki fragments. Referring to the replication fork shown in Fig. 1a, it is clear that the lagging strand must be synthesised discontinuously with *de novo* priming events. Electron microscopic evidence demonstrates that such priming events occur at least every few kilobases³⁷. In principle, the leading strand can be synthesised continuously, with no *de novo* priming events (except possibly at the origin of replication). However, early work on *E. coli* and T4³² showed that after a short pulse of ³H-thymidine, all the labelled DNA is found as slowly sedimenting chains in alkaline sucrose gradients (10S, corresponding to 1,000–2,000 nucleotides). Thus, the data suggested that both strands are synthesised discontinuously. Experiments with DNAI igase^{73,74} and DNA polymerase I mutants⁷⁵ reinforced this idea, since such mutants accumulate 10S fragments even after relatively long labelling periods, with little or no label found in large DNA. Demonstrations that *E. coli*⁷⁶ and phage P2⁷⁷ accumulate more fragments on the lagging strand than on the leading strand have been reconciled with the above observations by noting that the fragments on the leading strand are joined more rapidly than those on the lagging strand^{78,79}.

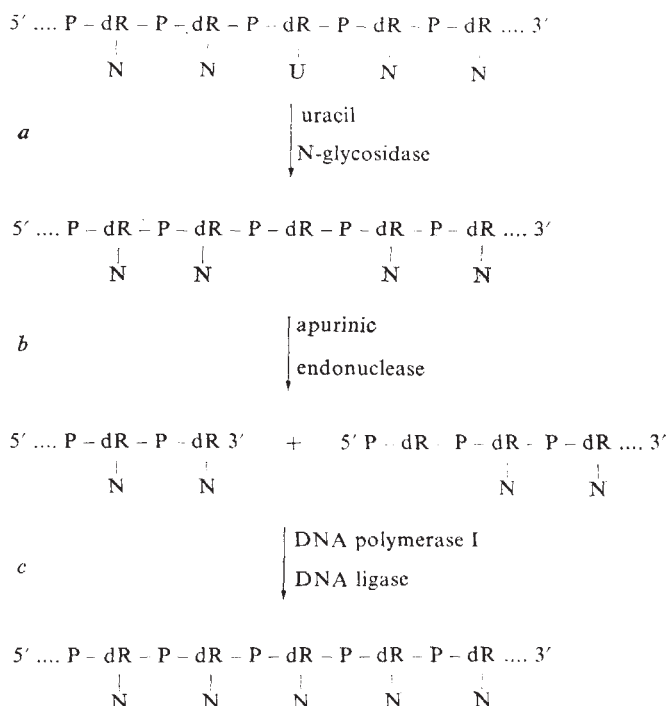


Fig. 3 A possible scheme for the excision-repair of uracil from DNA⁸². The phosphodiester backbone of the DNA is represented by ...P-dR-P-dR..., N represents any of the 4 normal bases (A, G, C, T) and U represents uracil. The repair process takes place on double-stranded DNA, but the complementary chain has been omitted for simplicity. *a*, Uracil *N*-glycosidase⁸³ removes uracil, leaving an intact phosphodiester backbone with a missing base. *b*, Apurinic endonuclease cleaves the DNA chain at the missing base⁸⁴. Recent data indicate that an alternative pathway, in which endonuclease V⁸⁵ recognises uracil and cleaves the DNA chain directly, is less active. *c*, DNA polymerase I and its associated 5'→3' exonuclease nick-translates⁸⁶, removing the damaged region and filling in the gap. Finally, DNA ligase joins the nick³⁴ to complete the process.

However, recent work reveals that not all DNA fragments labelled during a short pulse need arise from *de novo* priming events. In an *E. coli* mutant originally called *sof* (an acronym for small Okazaki fragments), pulse-labelling yields nascent DNA chains 5–10 times smaller than normal⁸⁰. It has now been demonstrated that *sof* mutants are identical in genotype and phenotype to *dut* mutants, deficient in dUTPase⁸¹. Such mutants have a higher than normal ratio of dUTP to TTP, and some dUTP is therefore incorporated into the DNA. The uracil is then rapidly excised by an excision-repair system which leaves temporary breaks in the DNA. Cells have a repair system to remove uracil residues from DNA, probably because of the frequent spontaneous deamination of cytosine in DNA to yield uracil, which is a potentially mutagenic event. A hypothetical scheme⁸² for the uracil excision-repair process is presented in Fig. 3.

In view of the above results with *dut* mutants, the question arises whether some Okazaki fragments in wild-type (*dut*⁺) strains are due to uracil incorporation and subsequent excision, rather than to true discontinuous synthesis. Even with a very active dUTPase there should be a finite level of dUTP available for polymerisation in the cell. In several *in vitro* DNA replication systems, low concentrations of dUTP strongly affect the size of the fragments synthesised (ref. 87; I. R. Lehman, personal communication). Conceivably then, all of the fragments on the leading strand (and some on the lagging strand) could be due to uracil misincorporation and subsequent excision-repair. If this were so, one might expect only 50% of a ³H-thymidine pulse-label to appear in Okazaki fragments in a uracil *N*-glycosidase mutant (see Fig. 3a). But pulse-labelling experiments have shown no difference between this mutant

(*ung*; ref. 88) and wild type (I. R. Lehman, personal communication; R. Sternglanz, unpublished results). Although results in the expected direction have been observed with the *ung* mutant in a cellophane disk *in vitro* system⁸⁷, the relevance of this result to the *in vivo* situation is unclear.

It should also be pointed out that other repair processes may similarly act on the nascent DNA to give rise to Okazaki fragments⁸⁹. For example, *E. coli* *dam* mutants (deficient in DNA adenine methylase) exhibit abnormally high recombination frequencies⁹⁰ (as do the *dut* mutants⁸⁰), presumably because of nicks and gaps in the DNA. Thus, the delayed methylation of nascent DNA could reduce its size. Because of such possibilities, the important question of what fraction of Okazaki fragments arise from *de novo* priming events remains unanswered, as does the question of whether the leading strand is synthesised continuously or discontinuously.

The priming mechanism for Okazaki fragments is complex

As discussed above, at least some of the Okazaki fragments on the lagging strand (and possibly on the leading strand) must be due to *de novo* priming events catalysed by an enzyme other than DNA polymerase. There are several important questions about such priming. Is the primer RNA and does it have a unique length and sequence? Are there unique start and stop signals for the primer on the template strand? Finally, what proteins are required for synthesis of the primer?

Definitive work on the priming of Okazaki fragments comes from a study of polyoma DNA fragments synthesised in an *in vitro* nuclear system. These fragments have been shown to have a 10-nucleotide-long stretch of RNA at their 5' ends, terminating in a 5'-triphosphate. The RNA is homogeneous in length but the sequence is not unique, not even at the RNA-DNA junction^{42,91}. Comparable results have been reported recently for human lymphocyte DNA fragments synthesised in an *in vitro* replication system⁹². However, it is still possible that an Okazaki fragment from a unique region (for example, isolated by hybridisation to a specific small polyoma DNA fragment) has a unique RNA primer sequence.

It has proved more difficult to demonstrate the presence of an RNA primer on *E. coli* Okazaki fragments. The most direct evidence comes from studies on fragments synthesised *in vitro* in toluenised cells; in that case a RNA-DNA junction has been chemically demonstrated⁹³. However, the evidence for RNA on *E. coli* DNA fragments synthesised *in vivo* is much more indirect. The initial experiments involving density shifts in Cs₂SO₄ equilibrium gradients⁹⁴ are now known to have been unreliable^{91,95}. The main evidence comes from experiments in which the nascent chains are treated with alkali, which hydrolyses the presumed RNA primer leaving a DNA chain with a 5'-OH terminus. The presence of the 5'-OH is then demonstrated by digestion of the DNA with spleen phosphodiesterase⁹⁶ or by phosphorylation of the DNA chain with polynucleotide kinase^{93,97}. However, it should be noted that the production of a 5'-OH terminus after alkali treatment of an Okazaki fragment does not prove the existence of RNA on the chain. For example, the presumed intermediate in the excision of uracil from DNA (Fig. 3b) might yield a 5'-OH terminus after alkali treatment, due to hydrolysis of the terminal deoxyribose from the chain via a 3'-4' cyclic phosphate intermediate^{98,99}. Direct demonstration of ribonucleotides on Okazaki fragments⁹⁷ is complicated by the difficulty in purifying the DNA free from traces of contaminating RNA chains. No one has been able to find on the 5' ends of *E. coli* Okazaki fragments the triphosphates that are found on fragments from mammalian cells^{91,92}.

As mentioned previously, *E. coli* DNA polymerase I mutants accumulate Okazaki fragments. They presumably do so because of a defect in a reaction termed nick-translation; in this reaction, DNA polymerase I can use its 5' to 3' exonuclease to remove the RNA primer from the 5' end of an Okazaki fragment, while simultaneously filling in the resulting gap with DNA⁸⁶. Those

mutants with the most severe defects in nick translation are actually conditional-lethal¹⁰⁰. Thus, one would predict that at least some of these mutants would accumulate the RNA primer. This prediction has been borne out in two laboratories using the aforementioned criterion of a 5'-OH terminus on the DNA chain after alkali treatment (ref. 97 and D. Denhardt, personal communication). However, two other laboratories have been unable to detect an RNA primer using the same or similar mutants (ref. 101; and B. Olivera, personal communication).

It is surprising that it has been so difficult to demonstrate directly the existence of RNA primers at the 5' end of Okazaki fragments synthesised *in vivo*. Some workers remain sceptical about the *in vivo* existence of RNA priming for this reason. On the other hand, it is not unreasonable to postulate that the primer is normally very rapidly removed *in vivo*, being preserved only in *in vitro* replication systems where the primer removal mechanism has been altered.

The enzymology of priming has been worked out in some detail for the *in vitro* replication of 3 different single-stranded circular bacteriophage DNA molecules; their conversion to the double-stranded form is carried out by the host *E. coli* replication proteins. Since these small DNA molecules do not have ends, DNA synthesis is absolutely dependent on the synthesis of a primer. Historically, RNA priming was first discovered in one of these systems¹⁰². Even though the priming reactions are carried out on specialised single-stranded DNA templates, they serve as possible models for the priming of Okazaki fragments at a replication fork.

The protein requirements for priming depend on the particular single-stranded bacteriophage DNA template used. For DNA from the filamentous bacteriophages, M13 and fd, the *E. coli* RNA polymerase responsible for most cellular RNA synthesis produces an RNA primer, initiating synthesis at a unique place on the single-stranded, circular DNA template^{102,103,104}. For bacteriophage G4 DNA, the primer is produced instead by the *E. coli* *dnaG* protein, an enzyme which, surprisingly, can polymerise either ribo- or deoxyribonucleoside triphosphates *in vitro* with nearly equal efficiency¹⁰⁵. This primer also begins at or near a unique site on the G4 DNA. It has recently been reported that the *dnaG* protein requires ADP in addition to the nucleoside triphosphates (ribo- or deoxy-) in order to form a primer on G4 DNA¹⁰⁶. For both M13 and G4 DNA, primer formation also requires enough *Eco* HD-protein I to completely cover the single-stranded DNA¹⁰³⁻¹⁰⁶.

Priming on bacteriophage ϕ X-174 DNA is much more complex. The *dnaG* protein synthesises the primer in this system as well, but at least 6 additional proteins are required: *Eco* HD-protein, *dnaB* and *dnaC* proteins, and additional DNA replication factors termed either X, Y and Z^{10,26} or j and n¹⁰⁷. These proteins, in an ATP-dependent reaction, are thought to form sites on ϕ X-174 DNA that can be recognised by the *dnaG* protein, which in turn synthesises the primer at these sites^{107,108}.

Since genetic evidence indicates that *dnaB*, *dnaC*, and *dnaG* proteins are all required for *E. coli* DNA replication, it seems likely that the mechanism of primer synthesis for *E. coli* Okazaki fragments is similar to that found for ϕ X-174 DNA. In fact, the *dnaG* protein has been indirectly implicated in the initiation of *E. coli* Okazaki fragments¹⁰⁹. Questions which remain to be answered for both ϕ X and *E. coli* primer formation include: (1) the exact role of the proteins whose action precedes the actual *dnaG* protein-mediated primer synthesis, and (2) the composition and sequence of the primer (RNA or mixed RNA-DNA copolymer).

The analogous RNA priming reactions detected in the bacteriophage T4 and T7 replication systems are as yet less well characterised; for discussions see refs 17, 20, 22, 29 and 30.

Prospects and conclusions

In spite of the reservations expressed above, the basic outlines

of DNA replication fork enzymology are now clear. DNA replication is a complex process, in which the asymmetric replication fork shown schematically in Fig. 1a moves along the parental DNA helix at rates of up to 1,000 nucleotides per second (in prokaryotes). In this process, multiple protein components, including DNA polymerases, helix-destabilising protein, DNA unwinding enzymes, DNA ligase, and RNA primer synthesising and erasing enzymes all have an important role. Several purified systems have been developed from prokaryotes which come close to reproducing this basic reaction *in vitro*.

DNA synthesis on the lagging side of the fork is clearly discontinuous, and the present evidence suggests that synthesis on the leading side of the fork may often be discontinuous too. One possibility is that lagging and leading strand syntheses are coupled, perhaps by a protein-induced folding of the DNA at the fork into an unique tertiary structure.

An unforeseen aspect of the replication process is the widespread use of ribonucleoside triphosphate hydrolysis energy. Presumably, each hydrolysis event drives an ordered allosteric change in protein conformation⁶⁴. Some of these changes generate force to aid in parental DNA helix unwinding^{27, 28, 56}, while others may exist only to facilitate protein-protein assembly reactions^{25, 110} and/or to enhance replication fidelity^{51, 52}.

In this connection, fidelity is a crucial aspect of the *in vivo* replication process which has not yet been adequately examined in any *in vitro* system. We have proposed above that the enormous replication fidelity which must be maintained *in vivo* (one error in 10^9 to 10^{10} bases copied) has placed severe restraints on possible replication mechanisms. In fact, much of the apparent complexity of the process may arise for this reason. Yet, present *in vitro* systems are routinely assessed as "faithful" by criteria (infectivity or restriction mapping) which would probably detect errors only if they exceed one part in 10^4 ! Thus, until a more sensitive assay for fidelity is used, these *in vitro* systems cannot be said to be truly "biological." They may even be missing central protein components which have little or no effect on synthesis rates, but which have major effects on fidelity.

In eukaryotes, replication forks must progress over bound nucleosomal histones, which may explain in part both the slower rate (100 nucleotides per s) and the smaller size of Okazaki fragments (a few hundred nucleotides) compared with prokaryotes¹¹¹. Replication over nucleosomes demands major conformational changes in the chromatin¹¹², which probably cannot be achieved *in vitro* without eukaryotic replication proteins. If only for this reason, the enzymology of a eukaryotic replication system needs to be worked out in detail, and a purified multi-component *in vitro* replication system developed, analogous to those discussed above from prokaryotes. Also to be learned from such a system is the source of the high fidelity in eukaryotic replication, especially if the eukaryotic DNA polymerases turn out to lack the 3' to 5' proofreading exonuclease of the prokaryotic enzymes^{111, 113, 114}.

We thank all those investigators who sent us preprints before publication. This work was supported by grant number GM 24020 from the National Institutes of Health, while R.S. was on sabbatical at the University of California.

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articles

‘Unmixing’ of the deep-sea record and the deglacial meltwater spike

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The Berger-Heath model of vertical mixing in deep-sea sediments provides a simple means of simulating an original input signal by ‘unmixing’ a signal found in deep-sea cores. When applied to an oxygen isotope record from the western equatorial Pacific, the possibility emerges that the entire ocean was covered by a low salinity water layer, during deglaciation.

DELETERIOUS effects of burrowing organisms on the resolution of the deep-sea record have been recognised for some time, and several mathematical models have been proposed to deal with signal distortions introduced by benthic mixing^{1–5}. The simplest one of these models² postulates a homogeneous layer at the sediment–water interface within which incoming particles are completely mixed with older sediment. In reality, of course, the intensity of mixing decreases with depth. In fact, mixing processes show a tiered structure, with intense mixing in an upper layer which is virtually homogeneous, and ‘lumpy’ mixing in a layer below. Sporadic displacement of sediment by large deeply burrowing organisms occurs to considerable depth. The upper two tiers of the entire mixed layer are of the order of 5 cm thick each, in the deep-sea carbonates studied, and the deep burrowing involves a layer at least 40 cm thick^{6,7}.

In spite of this complicated structure of the mixed layer, simple mathematical models can yield excellent results in simulating actual mixing processes. In particular, it can be shown that the distribution of particles within many ash layers found in deep-sea cores can be reconciled with a history of sudden emplacement of a thin homogeneous layer and subsequent simple exponential mixing^{8–10}.

It seems worthwhile, therefore, to try to run the mixing model backwards, in order to reconstruct a stratigraphic signal as it might have appeared had there been no benthic mixing to distort it. Here we derive a simple unmixing equation and apply it to an oxygen isotope record of deep-sea carbonates from the western equatorial Pacific, spanning the past 20,000 yr.

Unmixing equation

The pertinent differential equation is derived as follows: c_0 is the concentration before mixing of some stable tracer, within the accumulating sediment; the concentration in the mixed layer is c . For discrete particles with any kind of label (such as forams and

their isotopic composition) $c = nv$, where n is the particle concentration and v is the average tracer value.

When a thin layer of sediment, dl , accumulates, it brings with it $c_0 dl$ units of tracer per unit area. At the same time, the mixed layer moves up a distance dl and so loses cdl units of tracer. The concentration of the tracer in the mixed layer then changes by an amount,

$$dc = (c_0 dl - cdl)/m$$

dividing by dl , we get $c' = (c_0 - c)/m$; which means that the instantaneous gradient incorporated into the historical layer reflects the difference in the incoming signal (at the top of m) and the outgoing one (at its bottom), averaged over m .

Hence, to recover the original concentration c_0 from the record, we write

$$c_0 = c + c'm \quad (1)$$

This is the basic unmixing equation. In this model, the signal begins at the base of the mixed layer. When calculating sedimentation rates from a dated c_0 excursion, this shift has to be taken into account, that is, the distance to the sediment surface has to be reduced by m , before dividing by the age.

If sizeable changes occur in the proportion of signal-carrying particles, that is, in the proportion of the foraminifera whose isotopic composition is being studied, equation (1) has to be expanded. In the coarse sand fractions non-carbonate can be neglected, hence n , the particle concentration, is $s \times p$, where s is the grain size fraction and p the proportion of the species within that fraction.

The unmixing equation is applied first to n (that is, $\{s \times p\}$)

$$n_0 = n + n'm$$

and subsequently to $n \times v$

$$n_0 \times v_0 = n \times v + [n \times v]' \times m$$

and the solution sought is

$$\begin{aligned} v_0 &= n_0 \times v_0 / n_0 \\ &= (n \times v + [n \times v]' \times m) / (n + n'm) \end{aligned}$$

Table 1 Locations of box cores, depths from which they were raised, average carbonate content, and depth of the preservation spike at 14,000 yr b.p.¹²

Core	Latitude	Longitude	Depth (m)	CaCO ₃ (%)	Maximum preservation (cm)
ERDC 92 Bx	2 13.5'S	156 59.9'E	1598	83.6	26.5
ERDC 88 Bx	0 02.9'S	155 52.1'E	1924	88.2	21.5
ERDC 112 Bx	1 37.5'S	159 14.1'E	2169	84.3	23.5
ERDC 120 Bx	0 01.0'S	158 41.6'E	2247	85.0	26.0
ERDC 102 Bx	3 36.3'S	161 19.1'E	2306	87.1	17.5
ERDC 83 Bx	1 24.1'N	157 18.6'E	2342	83.0	23.5
ERDC 79 Bx	2 47.1'N	156 13.8'E	2767	84.4	19.5
ERDC 123 Bx	0 01.3'S	160 24.9'E	2948	83.1	26.5
ERDC 125 Bx	0 00.2'S	160 59.9'E	3368	82.8	21.5

Results from the first four and from the last four cores were pooled to derive the averaged signal in Fig. 1. Results from Core ERDC Bx 102 were rejected. Water depth is based on echo sounding, corrected for (assumed) regional differences in sound velocity.

where v_0 is the tracer signal, in our case oxygen isotope composition in ‰ PDB.

To test the efficacy of equation (1) (or its expansion) we can apply it to the ash data^{9,10}, where it will be seen that the saw-tooth-like frequency distributions of ash particles are readily converted to rectangular signal spikes, as expected. But, any noise in the record, that is, irregular excursions due to discrete burrowing, is immediately transformed into large erratic excursions. To avoid this it is necessary to collect several records of the same time interval, and to add them up in order to obtain a smooth average 'best' record.

Oxygen isotope record

Because of the considerable interest attaching to the climate and ocean dynamics of the past 20,000 yr, we have endeavoured to produce such an averaged oxygen isotope record for this period. Our cores are box cores from the western equatorial Pacific (see Table 1). The nine cores shown are well above the lysocline and are virtually free from dissolution effects, which distort the isotope record¹¹. The cores were sampled at closely spaced intervals, between 3 and 4 cm on the average. The isotopic composition of the planktonic foraminifer *Pulleniatina obliquiloculata* was determined by standard procedure, after establishing that the

isotopic signal of this species is similar to that of *Globigerinoides sacculifer*, in well-preserved sediment, except for an offset of 0.8‰¹¹. This offset reflects the fact that *P. obliquiloculata* grows its shell at somewhat greater depths than *G. sacculifer*, on the average.

Results of the determinations show a striking similarity of the oxygen isotope sequences in eight of the nine cores (see Fig. 1). Box core 102 does not follow the general pattern and is not considered further. Since all cores are from the same area, any idiosyncracies must be due to local disturbance.

We next average the eight acceptable sequences into one. Sedimentation rates are likely to differ somewhat, and isochrons must first be found, for proper addition. The exact dates of such isochrons are immaterial for this exercise.

We choose two such isochrons, besides the surface, for correlation. The first (13,000 yr b.p. according to ¹⁴C dating of Core 92 (ref. 11), is a point halfway between the late (isotopically light) segment of the high gradient transition from glacial to postglacial, and the segment of maximum preservation of the fine sand fraction (the preservation spike near 14,000 yr b.p.¹²). The second isochron (about 18,000 yr b.p.¹¹) is the point where the $\delta^{18}\text{O}$ values are presumed to be heaviest. The distance between the two isochrons in Core 92, which is ¹⁴C-dated¹¹ and in Core 112, where a very heavy isotopic value was obtained near 35 cm, was taken as a guide for determining where approximately this 18,000 yr b.p. level¹³ should be in the cores, in addition to the actual isotope determinations in each core.

The isochrons thus determined are shown in Table 2. A straightforward addition by core depth alone would yield a similar result: each core (except Bx 102) shows a small $\delta^{18}\text{O}$ minimum at the end of the steep transition segment, at almost identical depth, that is, near 20 cm. Our correlation procedure, therefore, besides being standard practice in deep-sea micropalaeontology, produces an acceptable match of the cores.

On the basis of this agreement as to depths in core (and not just sequence), we next construct two average isotope signals as a function of average depth with the $\delta^{18}\text{O}$ minimum centred on 20 cm (Fig. 1, two heavy lines). The first average signal is based on cores 92, 88, 112, and 120. The second is based on cores 83, 79, 123, and 125. Thus, the two average curves are derived completely independently. Their near-congruency is remarkable. We take this close agreement as further support for our correlation procedure. The median line between the two generalised signals (Fig. 1, fine line between two heavy lines) is our best estimate of a true oxygen isotope signal as would be recorded in an ideal core.

We consider this averaged signal to be more detailed and more reliable than any oxygen isotope record now available from deep-sea carbonates for this period. We realise also that this signal will have to experience some modifications as more data become available.

The result of applying our unmixing equation to this record is shown in Fig. 1. The reconstructed signal is represented by the shaded zone. The borders of the shaded zone show the possible amplitudes of the presumed original signal, for a mixed layer parameter m between 4 and 8 cm. This range for m has been established as reasonable for deep-sea sediment in general⁸ and also is indicated by unpublished results of closely spaced ¹⁴C

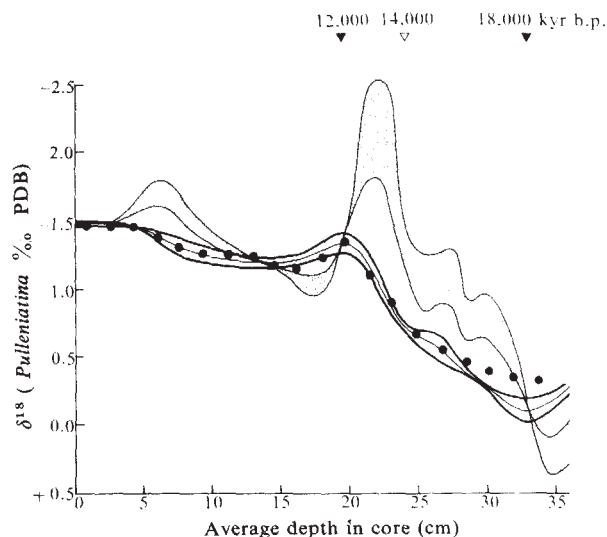


Fig. 1 Stacked and reconstructed oxygen isotope signal from eight box cores taken above the lysocline, on Ontong Java Plateau. Heavy lines: results of two separate stackings, of four records each. Centre line between two heavy lines: overall average signal. Shaded area: approximate estimated shape of original signal, using unmixing equation $c_0 = c + c'm$. Outer bound (large amplitudes): $m = 8$ cm. Inner bound (small amplitudes): $m = 4$ cm. The age scale refers to the actual signal, not the reconstructed one. Fix points are 12,000 yr b.p. for the meltwater anomaly, 14,000 yr b.p. for maximum preservation, 18,000 yr b.p. for the glacial maximum, based on ¹⁴C dating of core ERDC 92^{11,12}. The ●, represent an average isotope signal resulting from correlation by the preservation spike only (Table 1, last column) demonstrating the absence of bias in the combined isotope-preservation correlation procedure (see text).

Box core	Depth in core (cm)											
	0	5	10	15	20	25	30	35	40			
92	1.53	1.49	1.46	1.38	1.13	0.85	1.08	0.49	0.26	0.05		
88	1.68	1.61	1.53	1.44	1.50	1.69	0.88	0.91	0.78	0.69		
112	1.47	1.29	1.07	0.78	0.94	1.00	0.69	0.36	0.12	+0.19		
120	1.29	1.50	0.83	1.34	0.96	1.43	1.18	0.48	0.08	0.25		
102	1.39	1.45	0.95	1.00	+0.10?	0.50	0.54	.	0.58			
83	1.45	1.40	1.56	1.26	1.19	1.46	0.83	0.67	0.36	0.24	0.33	
79	1.47	1.38	1.32	1.46	1.17	1.25	1.29	0.56	0.40	0.34	0.43	
123	1.33	1.17	1.38	1.25	1.36	1.10	1.22	0.23	0.16	0.65?		
125	1.53	1.81	1.47	1.18	1.25	1.34	1.50	0.70	0.43	0.16	0.21	
	‘13 kyr b.p.’								‘18 kyr b.p.’			

Fig. 2 Oxygen isotope composition of *Pulleniatina obliquiloculata* in nine box cores from Ontong Java Plateau above the lysocline (~ 3,500 m). Decimal points indicate position of sampling midpoint. All values in $\delta^{18}\text{O}_{\text{‰}}$ PDB (negative, unless marked +). Stippled lines indicate estimated positions of two isochrons (~ 13,000 yr b.p. between (estimated) maximum anomaly of product [$c \times c'$] and centre of maximum preservation; and ~ 18,000 yr b.p. at maximum value of $\delta^{18}\text{O}$).

dating of Core 92, by T. H. Peng. The larger m produces the larger amplitude, as seen from equation (1).

The resulting reconstructed signal, that is, the shaded field in Fig. 1, has some interesting features, the outstanding one being the pronounced $\delta^{18}\text{O}$ minimum (near 12,000 yr b.p.). One might argue that the amplitude of this minimum is partly due to our method of correlation, in which the isotope signal itself had one-half weight for determination of the 13,000 yr b.p. isochron. This objection, raised by N. J. Shackleton, in private discussion, is entirely justified. We have, therefore, re-done the correlation using only the centre of the preservation spike as a guide. The resulting average isotope signal is shown in Fig. 1, as a string of heavy dots. It is obvious that the outcome of an unmixing exercise on this signal would be quite similar to the reconstructed signal given.

In showing a pronounced $\delta^{18}\text{O}$ minimum near 12,000 yr b.p. and also in other details, our reconstruction resembles closely the raw oxygen isotope records found by Kennett and Shackleton¹⁴ and by Emiliani *et al.*¹⁵ in the Gulf of Mexico. Their signals are derived from cores with high sedimentation rates which are much less susceptible to distortion by benthic mixing than ours. Emiliani *et al.* date the prominent $\delta^{18}\text{O}$ minimum near 12,000 yr b.p., a date that agrees with ours and with the estimate by Kennett and Shackleton. Hence, we are reasonably confident that we are seeing a worldwide phenomenon, of which the Gulf of Mexico anomaly is a special regional manifestation. (Note that the age scale in Fig. 1

refers to the actual signal, not the reconstruction, so that the 12,000-yr date shown coincides with the original $\delta^{18}\text{O}$ minimum.)

A worldwide phenomenon

What is this phenomenon? The Gulf of Mexico results have been interpreted as showing an influx of meltwater from the Laurentide Ice Sheets^{14,15}. This influx would strongly decrease the $\delta^{18}\text{O}$ values of the upper water layer, and hence of the shells of the foraminifera living within it.

We propose that a similar effect may exist for the entire world ocean. Our interpretation supports the hypothesis of Worthington¹⁶, who postulated that the rapid influx of meltwater to the world ocean must have resulted in a low salinity upper water layer, forming a lid on the ocean. Below this lid, which prevented vertical mixing, CO_2 would have accumulated and this CO_2 enrichment would have led to increased dissolution of the shells of foraminifera on the sea floor¹⁶.

The increase of dissolution postulated by Worthington has been found, and occurs exactly at the point predicted¹². On renewed mixing, any deep-trapped CO_2 should have been released to the atmosphere. A rapid warming might be expected at this point. There is evidence that the warming at the beginning of the Holocene, which led to the Hypsithermal was very rapid^{17,18}. In our scenario, this warming event is the one recent geological analogue of the present large-scale release of industrial CO_2 to the atmosphere. Its course and its effects on the biosphere should hold great interest for future investigation.

This research was supported by the US NSF (grant OCE 75-04335) and by the Office of Naval Research (contract USN N00014-75-C-0152). The Stable Isotope Sediment Laboratory was set up with US NSF support (DES 75-04497, to W. H. Berger and M. Kastner) and with support from the Scripps Industrial Associates and from Chevron Oil, California. We thank E. Vincent for supervising sample preparation and for discussions. The box coring device was provided by R. R. Hessler, and G. Wilson assisted in taking the cores.

Received 23 May; accepted 25 July 1977.

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Plate boundary within Tjörnes Fracture Zone on northern Iceland's insular margin

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The current North American-Eurasian plate boundary on Iceland's northern insular margin is defined. Overlapping rift zones and en echelon post-glacial volcanic eruptions oblique to the apparent transform direction of the Tjörnes Fracture Zone suggest that the fault is in a transient deformational stage.

Iceland represents an anomalous segment of the Mid-Atlantic Ridge that remains unexplained by the simple elements of plate-tectonic theory. The abnormal build-up of the Iceland platform also coincides with the pronounced change of the ridge direction. Although the history of rifting over Iceland is complex, the zones of neovolcanic and tectonic activity now appear fairly well defined^{1,2}.

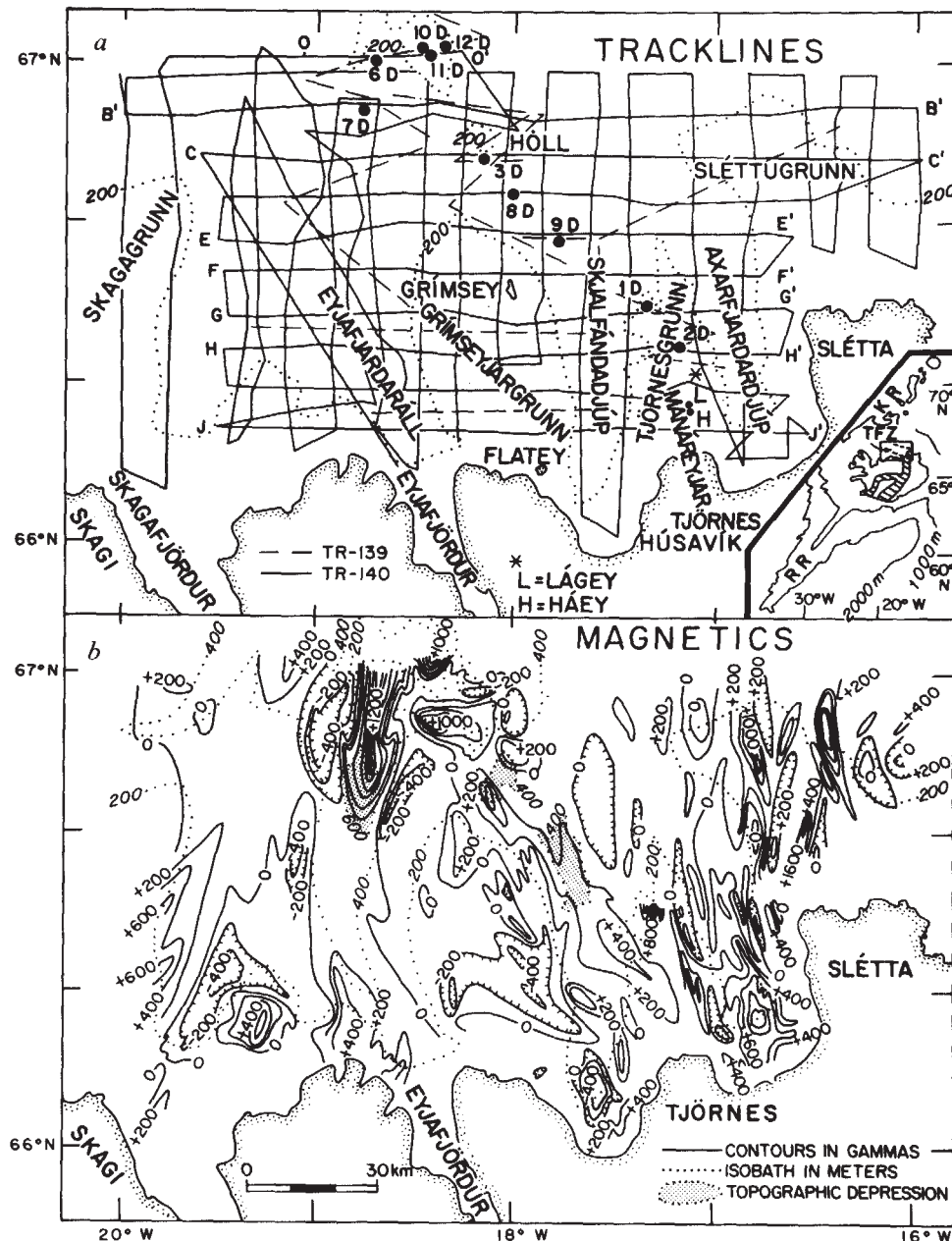


Fig. 1 Insular margin, northern Iceland; a, locations of R/V Trident's TR-140 tracklines and TR-139 tracklines and dredge stations; b, magnetic anomalies. On index map KR, Kolbeinsey Ridge; RR, Reykjanes Ridge; TFZ, Tjörnes Fracture Zone.

This report focuses on the offset zone between the Kolbeinsey Ridge^{3,4,5} and the north-east neovolcanic zone (NENZ) (ref. 6) of northern Iceland's insular shelf, commonly referred to as the Tjörnes Fracture Zone (TFZ) a right-lateral transform fault^{3-5,7-9} (Fig. 1a). Not only is the existence of the transform fault questioned^{2,10} but proponents^{4,5,8,9} acknowledge that topographic expressions paralleling the transform direction fail to control the offset zone.

We report here new evidence derived from a detailed field investigation involving bathymetry, magnetics, seismic reflection and rock dredging on the insular margin. The survey was conducted during the University of Rhode Island's R/V Trident cruises 139 and 140, in summer of 1973. The ship's tracks shown in Fig. 1a were controlled by satellite navigation.

Our results reveal that morphologically and tectonically the TFZ is dominated by three N-S striking lineaments, namely Kolbeinsey Ridge—Eyjafjardaráll Trough Complex (KRETC), Kolbeinsey Ridge—Skjálfandadjúp Trough Complex (KRSTC) and the Axarfjardardjúp Trough. These features display remarkable continuity across the shelf and overlap each other towards the north-west. The three troughs are distinguished by faulting with post-glacial volcanic activity occurring within

both Eyjafjardaráll and Skjálfandadjúp rifting zones but not along their entire lengths (Fig. 2). In contrast the zones of recent volcanic activity are arranged in a NNW-SSE *en echelon* fashion from the Kolbeinsey Ridge towards the axis of the NENZ (Fig. 2) and thus do not imbricate significantly as the rift zones do.

Kolbeinsey Ridge—Eyjafjardaráll Trough Complex

The KRETC is governed by N-S dip-slip faulting. On the north the ridge is symmetrical, remarkably regular, and broken only by the small persistent rift valley that is offset westward from the crestal axis (Fig. 3, profile O). A strong positive magnetic anomaly is aligned along the valley trend, flanked on the west by a weaker but equally well-defined negative anomaly (Fig. 1b). Small eruptive fissures typify the graben's floor which is shown by dredging to consist of fresh pillow basalts (Fig. 2, Table 1, station 6 and 7). Onlapping sediments thicken away from the axis as expected for normal spreading ridges. Southwards the character of the ridge is altered significantly: the elevation decreases as the width increases even though the

Table 1 TR 139 rock dredging across Tjörnes Fracture Zone

Station	Latitude and Longitude	Depth (m)	Feature	Recovery	Inference
1D	66°30.5'N 17°20.5'W	65–80	Flat-topped seamount; NW Tjörnesgrunn	Fresh glassy pillow basalt fragments	Post glacial submarine eruption (possibly 1868)
2D	66°25.5'N 17°10'W	16–25	Mánáreyjar ridge crest; Lágey	Poorly sorted palagonitized tuff fragments	Not far from source vent
3D	66°48.5'N 18°09'W	180–205	Kolbeinsey Ridge— Skjálfandadjúp Trough Complex; Höll Seamount	Rounded and jointed cobbles; oxidised scoriaceous vesicular basalt	Subaerial or very shallow depth submarine eruption, near vent, wave erosion and subsidence—post glacial
6D	67°00.5'N 18°42.5'W	270–310	Kolbeinsey Ridge— Eyjafjardaráll Trough Complex—eruptive fissure on graben floor	Fresh glassy pillow basalt	Typical submarine ridge eruption—post-glacial
7D	66°54.5'N 18°46.5'W	400–410	Kolbeinsey Ridge— Eyjafjardaráll Trough Complex—eruptive fissure on graben floor	Fresh glassy pillow basalt with brown crust	Typical submarine ridge eruption—post-glacial
8D	66°44.5'N 18°00.5'W	340–350	Kolbeinsey Ridge— Skjálfandadjúp Trough Complex—small graben	Tuff fragments, 3 small pebbles of massive basalt, indurated balls of mud with glass shards (one surrounded with a small film of lava)	Subaerial volcanic material with some recent submarine volcanic activity
9D	66°38.5'N 17°47'W	180–210	Kolbeinsey Ridge— Skjálfandadjúp Trough Complex—isolated seamount within graben complex—with sediment disturbance	Large vesicular basalt, slightly glassy—small fresh highly vesicular pillows with palagonite crust; rounded cobbles—small fist-size grabbo nodules	Post glacial subaerial or shallow submarine eruption—inconclusive
10D	67°02.5'N 18°28.5'W	65–80	Kolbeinsey Ridge— Skjálfandadjúp Trough Complex. Large flat-topped volcano with two distinct terraces at 55 m and 90 m	0.3 m rounded vesicular basalt	Post glacial subaerial eruption—wave erosion
11D	67°01.5'N 18°26'W	90–100	Same feature as 10D	Rounded vesicular basalt; banded tuff, near vent, few erratics	Post glacial or inter-glacial subaerial eruption, near vent, wave erosion, subsidence
12D	67°02.5'N 18°22'W	90–100	Same feature as 10D (90 m terrace)	Cobbles and pebbles of vesicular basalt; 1 limestone fragment	Post glacial or inter-glacial subaerial, volcanic island; surf zone type deposit

acoustic basement configuration and sediment accumulations remain symmetrical about the ridge axis (Fig. 3, profile B). The subsiding effect serves to promote fracturing. The south-eastern ridge extension, marked by peaks and small grabens maintains its ridge profile but the southern ridge branch gradually changes to a linear depression. In the Eyjafjardaráll Trough at 66°40'N, the coincidence of intersecting faulting, the limit of axial magnetic stripes and sediment deposits⁵ coupled with the site of the closed 40 mgal free-air gravity anomaly contour¹¹ define a structural-volcanic discontinuity (Fig. 2). Southwards the trough is bordered and apparently controlled by N–S dip-slip faults which at the trough's shoreward end bend toward the SE to join the WNW Húsvík fault trend⁹. Sediments more than 0.3 s thick fill the trough and no evidence of volcanic activity is observed. Thus the 66°40'N structural-volcanic discontinuity clearly represents the southernmost extent of spreading and rifting in the TFZ which is in direct continuity with the present Kolbeinsey Ridge spreading axis.

Kolbeinsey Ridge–Skjálfandadjúp Trough Complex

The SE curving KRSTC shows numerous dip-slip faults that follow the trend (Fig. 2). The south-eastern ridge extension contains a series of discontinuous volcanic peaks, including Höll, and small grabens along the trend that disappear at 66°49'N. Only the south-eastern end of a negative anomaly

band is evident due to its disruption by a strong positive anomaly west of Höll (Fig. 1b). Onlapping sediment deposits typify the extension's eastern flank (Fig. 3, profile C). The adjoining Skjálfandadjúp's borders are controlled by normal faulting except over its southernmost reach where downwarping seems to govern its western flank (Fig. 2). A distinct NW–SE trending positive anomaly can be traced from Höll to Tjörnesgrunn (Fig. 1b). The north-western half of this anomaly overlies the trough's floor. East and south of Grímsey the trough turns to the south but the positive anomaly maintains its SE strike, cutting across the trough's eastern wall and intersecting the western flank of Tjörnesgrunn at a high angle. The most intense faulting occurs north, east and south-east of Grímsey (Fig. 2). The direction and style of faulting fail to support the presence of a major WNW right-lateral strike-slip fault in this area previously inferred¹². We also feel that the distribution and precision of earthquake epicentre locations are insufficient to resolve the two interpretations (Fig. 2). Furthermore the trough's northern section contains numerous submarine eruptive features where little or no sediment is accumulating (Fig. 2, Table 1, stations 8 and 9). It is clearly evident that volcanic activity in the Skjálfandadjúp rifting zone is restricted to the segment north and north-east of Grímsey. Both subaerial and fresh submarine volcanic products, apparently post-glacial, are present. Rocks from Höll Peak and southwards are dominated by vesicular and scoriaceous basalts rounded by wave action suggesting subsidence of the ridge extension (Fig. 2,

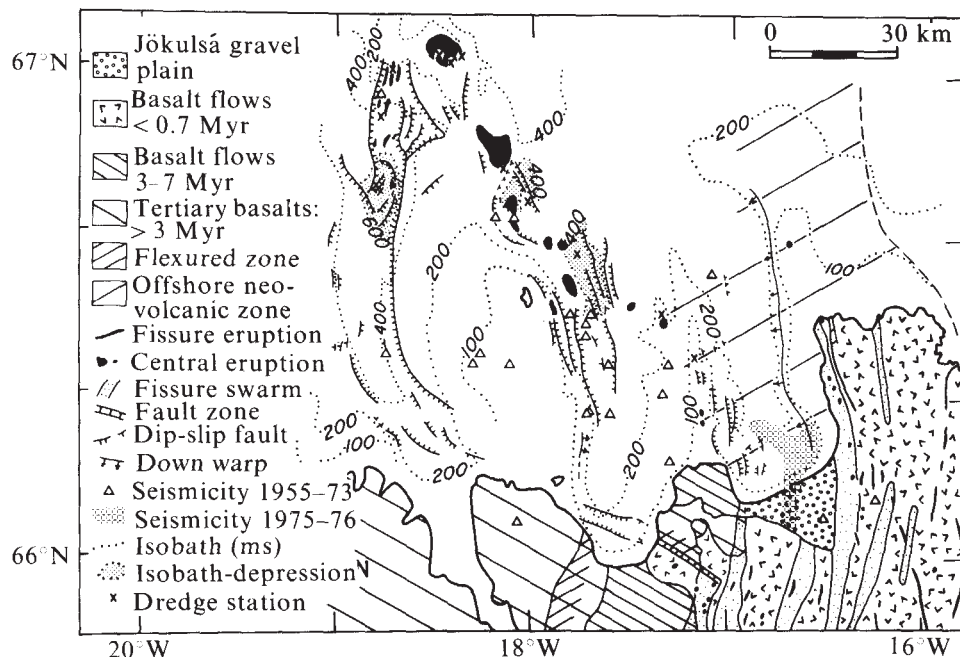


Fig. 2 Summary geology map. Earthquake epicentre locations (refs 13, 21); 1964-73 epicentres from Preliminary Determination of Epicenters Monthly Listing published by US Coast and Geodetic Survey and successor organisations through 1972 and jointly by NOAA/USGS thereafter.

Table 1, stations 3, 10, 11 and 12). South of 66°31'N no recent volcanic activity is observed as sediment covers the trough's floor reaching a thickness of 0.13 s towards the coast.

Axarfjardardjúp Trough

This trough is a downwarp modified by faulting, rifting and volcanism (Fig. 2). The predominant direction of the dip-slip faults is parallel to the trough's long axis. Normal faulting is associated with the eastern flank of the apparently older Mánareyjar volcanic chain and can be traced southwards to the Tjörnes Peninsula's eastern border scarp¹³. The trough's magnetics show a series of narrow, elongated anomalies which differ markedly from the Kolbeinsey Ridge anomaly bands (Fig. 1b). Notably the western edge of this anomaly series strikes NNW-SSE and diagonally crosses the Tjörnesgrunn trend⁶. Recent undeformed sediments up to 0.1 s thick, partially or completely cover the trough's floor (Fig. 3, profiles H and J). Although no morphological indications of volcanism are manifest and no search for evidence of recent volcanic activity was possible due to time limitations, pre-depositional rifting is distinctly revealed at the trough's southern end alongside Tjörnes Peninsula (Fig. 2 and Fig. 3, profile J). Moreover, since our survey's completion, significant recent rifting¹² is reported in the same part of the trough covered by our seismic reflection lines but lies immediately eastward of the pre-depositional rift zone. Hence recent rifting occurs in the offshore NENZ⁶ and is not found on the Mánareyjar chain as indicated previously².

Subdued shelf ridges

The three tectonic trends discussed above are separated by Sléttugrunn, Tjörnesgrunn and Grimseygrunn (Fig. 1a). On the east Sléttugrunn is the northward lobate projection of Sléttá Peninsula (Fig. 1a). It is characterised by the same magnetic anomalous pattern observed in the Axarfjardardjúp Trough (Fig. 1b). Alignment of these anomalies with fissure swarms on land as well as their general N-S strike and high magnetic intensity suggest an offshore continuation of the NENZ (ref. 6). Some volcanism is revealed by the presence of a single cone and apparent flows, indicated by the lack of sub-bottom penetration, that cover the surface immediately seaward of the Sléttá Peninsula but no seismicity is reported⁹ (Fig. 2). Thus volcanically and tectonically most of Sléttugrunn may represent a presently dormant zone.

Tjörnesgrunn is a tapering N-S submarine extension of the Tjörnes Peninsula that includes the Mánareyjar volcanic

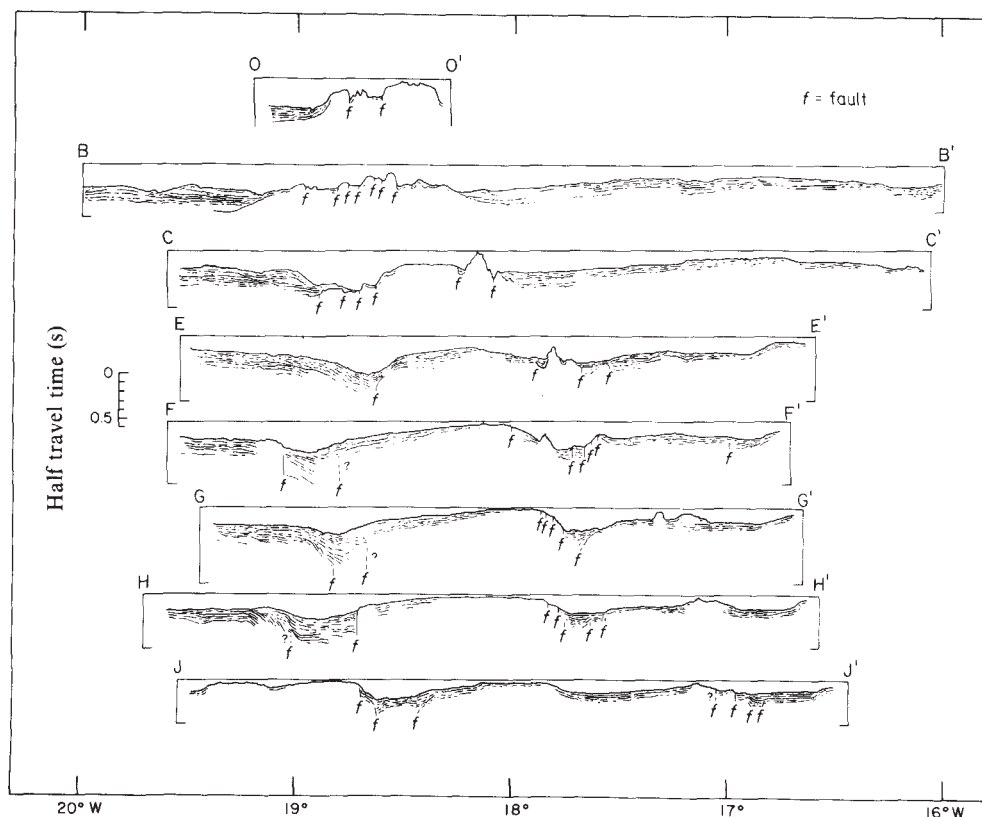
chain (Fig. 1a). Its eastern flank, partly fault controlled, is steeper and more irregular than its western flank. The western edge of the magnetic anomaly series that distinguishes Axarfjardardjúp and Sléttugrunn, passes beneath the ridge's outer reach (Fig. 1b). No evidence of recent submarine volcanic activity is encountered along the N-S Mánareyjar ridge crest, which is probably formed of relatively older subaerial volcanics. Only palagonitized tuff and interbedded vesicular basalt pebbles are dredged from the crest at the ridge north of Lágey (Fig. 1a and Table 1, station 2). The material is similar to that found on Háey, or overlapping the grey basalt flow of Lágey islets. Near the NW end of Tjörnesgrunn, however, (Fig. 1a, Table 1, station 1) very fresh glassy vesicular pillow basalts is recovered from a flat-topped seamount. The seamount definitely represents a recent submarine eruption at very shallow depth, perhaps representing the 1868 volcanic activity reported in Icelandic history. This volcanism, lying along the NENZ's western border may suggest another small rift segment linking the general NNW *en echelon* pattern of rifting and latest volcanic activity that occurs in the Skjálfandadjúp.

Grimseyjargrunn is a tabular block that shows no internal acoustic reflectors (Fig. 1a and Fig. 3, profiles F, G, H and J). The block is negatively magnetised crust except for its northern and southern edges which display weak positive anomalies (Fig. 1b). The ridge's northern and eastern walls descend steeply into Skjálfandadjúp while its western flank with on-lapping sediments slopes gently westwards into Eyjafjardaráll. The westward dip is the same as that reported for Tertiary flood basalts of the adjacent coastal area⁹. Grimsey Island, laying atop of Grimseyjargrunn, also consists of SW dipping plateau basalts with a suggested age younger than mid-Gauss⁹.

Húsavík faults

The Húsavík faults⁹ are extended west-north-westward across Skjálfandadjúp's southernmost reach (Fig. 2). Between Húsavík and Eyjafjardaráll, the axis of a gravity low¹¹ lies immediately north of the apparent trace of the offshore fault zone. At the southern end of Eyjafjardaráll, the centre of the gravity low is elongated WNW-ESE and the trough's faults bend towards the south-east. These deviations from the N-S trend may reflect strike-slip dislocations along the seaward extent of the Húsavík faults between 4-1 Myr age⁹. Thus the Húsavík fault zone is unrelated to present rifting of the Mid-Atlantic Ridge as activity along these faults is believed to have diminished about 1 Myr ago¹⁴.

Fig. 3 Line drawings of selected seismic reflection profiles. For locations see Fig. 1a. *f*, Fault.



Discussion

From these preliminary results, we infer that no transverse tectonic features common to transform faults^{15,16} are recognised over the TFZ with the exception of the Húsavík faults which are believed to be presently inactive and unrelated to the plate boundary. Instead the TFZ is characterised by three distinct and overlapping tectonically active zones associated with the KRETC, KRSTC, and Axarfjardardjúp Trough (NENZ). Seismicity shows no simple and direct relationship with these active zones except for the post 1973 earthquakes at Axarfjardjúp's southern end¹² (Fig. 2). Recent volcanic activity along eruptive fissures between the Kolbeinsey Ridge and NENZ occurs in NNW-SSE *en echelon* manner (Fig. 2) and defines the most recent plate boundary between Eurasia and Greenland. Practically no overlap of volcanic activity is found except perhaps immediately south of 67°N where Eyjafjardaráll and Skjálfandadjúp connect with the Kolbeinsey Ridge. The *en echelon* arrangement of recent eruptive fissures is further corroborated by magnetic anomaly lineaments and to some extent by the distribution of recent earthquake epicentres in Axarfjardardjúp Trough¹³.

The surface structural character of the TFZ revealed in our survey may be compared profitably with the transform tectonic expressions produced by a simple experimental clay model for the transient stage of discontinuous deformation¹⁷. During this stage the azimuth of fault traces develops oblique to the offset direction. Clear similarities are found but discrepancies are also evident. Analogies include: (a) the assumed displacement direction being roughly 65° with respect to the normal faults of the Kolbeinsey Ridge and Axarfjardardjúp Trough (NENZ); (b) *en echelon* dip-slip faults and eruptive fissures in Skjálfandadjúp striking 30°–40° relative to the presumed displacement trend; (c) intersecting rift zone and oblique fault systems forming crude diamond-shaped blocks in the Kolbeinsey Ridge-Eyjafjardaráll Trough transition. Significant dissimilarities are that *en echelon* oblique faults are not distributed uniformly across the assumed displacement zone, major strike-slip movement is not documented along these faults although near Grímsey strike-slip motion has been determined recently from a fault plane solution¹² and no intersecting fault

pattern is found where the inferred activity meets the NENZ. Despite these discrepancies we believe that the arrangement of volcano-tectonic features within the TFZ is best explained by discontinuous transformation as expressed by the clay model¹⁷. Perhaps the transient stage is somewhat mature judging from the 30°–40° angle that faulting and eruptive fissures form with the presumed displacement of the transform fault.

These types of transient or leaky transform faults are also noted south of Iceland, the Afar, and south of the Azores Platform at 37°N (refs 17, 18). The three areas occur at the loci of proposed mantle plumes and major directional changes of the mid-ocean ridge. Independently, Burke *et al.*¹⁹ suggest that mid-ocean ridges located near mantle plumes will tend to jump back over the plume during a transient stage thus generating multiple spreading ridge axes and consequent fracture zones, overlapping to some extent in space and time. Recent detailed spatial and temporal analyses of active rift zones throughout Iceland^{2,20} seem to indicate such rift jumping. Our proposed transient stage of discontinuous deformation for the TFZ, therefore, seems to be consistent with such a model. Further Walker's² conjectured *en echelon* active rift zone configuration for the TFZ, based on meagre data, is essentially confirmed.

Finally, tensional fissures removed from the major plate boundary are clearly documented in the clay model experiment for the transient stage of discontinuous deformation¹⁷. Therefore we suggest that the active eruptive centres along Sléttá's western coast (Fig. 2) which remain unexplained² as well as the recent linear submarine volcanic zone of the KRETC are identical features. As such these kinds of eruptive fissures may have a similar origin to that proposed for the Vestmanna Islands¹⁷. In these cases the fissures are considered to lie along prolongation of normal faults that extend into the plate for limited distances on diagonally opposite sides of the offset.

We acknowledge support (NSF Grant DES 72-01705) and are grateful to Iceland's National Research Council for permitting the field work to be undertaken.

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Amino-terminal fragments of *Escherichia coli* lac repressor bind to DNA

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The N-terminal fragments (residues 1–51 and 1–59) obtained by selective tryptic cleavage of native lac repressor retain the ability to bind DNA. These fragments (headpieces) are monomeric and form complexes which resemble those of tetrameric repressor with non-operator DNA. But, they do not show the high specificity of repressor for operator sequences. The DNA binding has been demonstrated by filter-binding assay as well as in solution using absorption, circular dichroism, and fluorescence measurements.

There is overwhelming genetic and biochemical evidence to indicate that the integrity of the amino-terminal segments of the *Escherichia coli* lac repressor subunits is a necessary requirement for the weak binding to non-operator DNA as well as the highly specific and strong binding to operator DNA^{1–6} (for reviews and discussion, see refs 7–9). Geisler and Weber¹⁰ have reported the selective cleavage by trypsin of the N-terminal region of repressor at Arg₅₁ and Lys₅₉ so as to liberate approximately equal amounts of 'short' and 'long' headpiece peptides (designated here as SH and LH) comprising residues 1–51 and 1–59. The tetrameric core no longer binds DNA (ref. 3) but the headpiece preparations retain affinity for phosphocellulose and DNA-cellulose, a criterion for nonspecific DNA binding^{7,9}.

The aim of this study was the initial characterisation of the DNA-binding properties of the two headpiece fragments and their correlation with known features of native repressor. The presumption that the characteristics of headpiece are related to those of the parent repressor and are thus biologically relevant is based on the following considerations: (1) isolated headpiece possesses secondary structure revealed by circular dichroism¹⁰. (2). The pattern of susceptibility to trypsin digestion is the same for isolated headpiece and the corresponding region in native repressor. Thus, in 1 M Tris-HCl, pH 7.5, 30% glycerol, repressor is cleaved so as to generate headpiece but the latter is not susceptible to further digestion¹⁰. We have established that the isolated headpiece is also resistant to trypsin in the same conditions (data not shown). In 0.05 M Tris buffer, however, both isolated headpiece and the corresponding region in intact repressor rapidly undergo further proteolysis to peptides (data not shown). (3). The capacity of repressor to bind DNA nonspecifically is transferred to β -galactosidase in chimaeric molecules produced by certain gene fusions between the *i*- and *z*-genes of the *lac* operon⁶. These molecules carry about 59–80 residues of the N terminus of repressor. Further evidence for the close relationship between the properties of the isolated headpiece and those of native repressor was obtained in the present study.

In interpreting these findings, one should note the close correlation between the operator-specific and the nonspecific modes of binding of lac repressor to DNA and the important role ascribed to the sequence-independent interactions in the regulation of repression^{6–9,11}.

Determination of DNA binding by filter assay

The two headpieces LH and SH can be separated on DNA-cellulose columns (Fig. 1a). SH elutes before LH, as might be expected due to its smaller size and charge. Both fragments are active in binding assays based on the retention of protein-DNA complexes on nitrocellulose filters (Fig. 1b). The markedly sigmoidal binding isotherms denote the existence of real (or apparent) co-operativity, a feature not seen with native repressor. While the binding of DNA is quantitative at high concentration of either headpiece, the points for 50% retention and the corresponding slopes differ markedly (0.8 μ M and 3 μ M for LH and SH, respectively). The mixed headpiece preparation shows an intermediate behaviour consistent with the superposition of LH and SH properties, the former being dominant.

Additional features revealed by the filter assay are given in Table 1. In standard conditions, various natural and synthetic DNA's are bound in a similar fashion. Thus, headpiece does not demonstrate a sequence-specific (operator) interaction with DNA, in contrast to the parent repressor. Denaturation of the DNA slightly potentiates binding by headpiece while fragmentation by sonication diminishes it only to a limited degree (note also the large difference in chain lengths between the phage DNA and poly d(A-s⁴T) used in Table 1). Salt manipulations lead to complex and somewhat DNA-dependent effects. Eliminating Mg²⁺ or raising the KCl concentration increases binding at low headpiece concentrations but reduces the maximal extent of retention, especially in the case of phage DNA. One is undoubtedly perturbing the thermodynamic¹⁷ and kinetic⁷ parameters governing the formation of the binary complex and/or its interactions with the filter. Finally, the kinetics of association and dissociation are relatively rapid. With poly d(A-s⁴T) and headpiece at 50 nM and 2.5 μ M, respectively, a filterable complex is formed within 10 s (a value which only sets a lower limit for k_{on} of about 10⁴ M⁻¹ s⁻¹). Similarly, challenging a preformed complex with an 100-fold excess of non-radioactive poly d(A-s⁴T) leads to dissociation within seconds. Taken together, these data establish that the headpiece of lac repressor possesses the inherent property to bind DNA and that the observed phenomena are not due to contamination with residual functional repressor.

Spectroscopic measurements using poly d(A-s⁴T)

Four of the eight tyrosine residues in each repressor subunit are located in the N-terminal region (residues 7, 12, 17 and 47) (ref. 16). Genetic analysis of repressor mutants has shown that Tyr₁₇ is essential for specific operator binding¹⁸. The spectral properties of the tyrosines are particularly suitable for study since no other chromophoric amino acid side chains are present in the headpiece. Figure 2A(a) shows the absorption spectrum of LH which corresponds closely to that of unionised unperturbed (exposed) tyrosine. Thus, the buried and ionised (or hydrogen-bonded) tyrosine residues observed in intact repressor by ¹⁹F nuclear

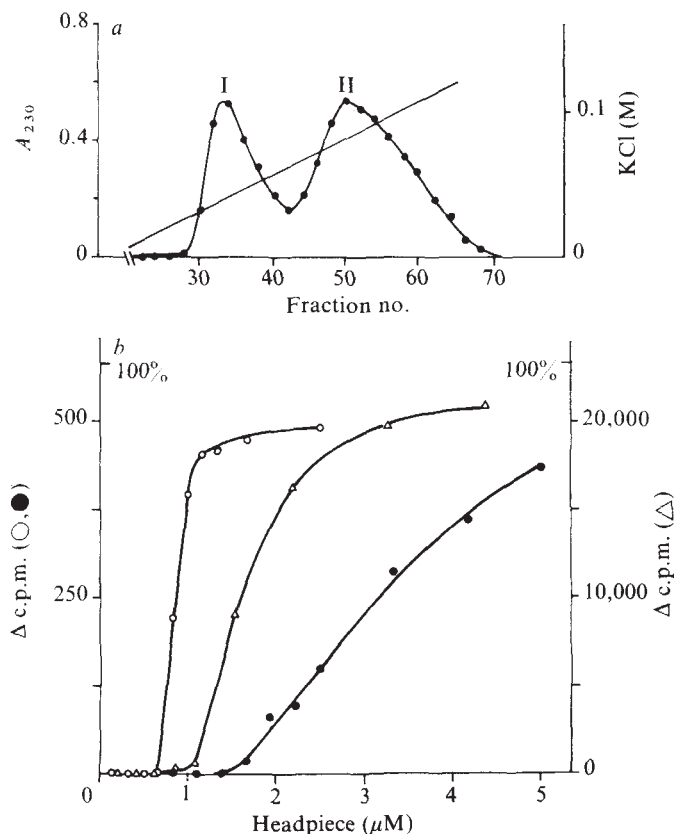


Fig. 1 Purification and DNA-binding properties of *lac* repressor headpieces. *a*, Separation of LH (long headpiece: residues 1–59) and SH (short headpiece: residues 1–51) on a DNA-cellulose column. Tryptic digests of repressor were prepared and purified by chromatography on Sephadex G-150 and G-25 according to Geisler and Weber¹⁰. The headpiece mixture was applied to a native calf thymus DNA-cellulose column¹² equilibrated with 50 mM Tris-HCl, pH 7.8 (4 °C), 1 mM EDTA, 5% glycerol and eluted with a 300 ml linear gradient of buffer containing 0–0.15 M KCl. Peaks I and II were identified as SH and LH, respectively, by amino acid analysis and C-terminal determination with carboxypeptidase B. The molecular weights of SH and LH are 5,615 and 6,395, respectively, from the amino acid composition. *b*, DNA binding by the two headpiece preparations and their unfractionated mixture. Slight modifications of the standard assay¹³ for the operator binding activity of repressor were used to increase retention and reproducibility: the 25-mm Millipore HAWP (0.8- μ m pore size) filters are supported on a glass holder, the outlet of which is filled with liquid and attached to a peristaltic pump. The binding buffer (BB) (0.6 ml) contained 10 mM KCl, 10 mM Tris-HCl, pH 7.4, 3 mM magnesium acetate, 0.1 mM EDTA, 5% dimethyl sulphoxide (DMSO), 0.1 mM dithiothreitol (DTT), and 50 μ g ml⁻¹ BSA. ³²P-labelled λ phage DNA (see Table 1) was present at a nucleotide concentration of 0.9 μ M (specific activity 1300 c.p.m. nmol⁻¹ in the case of LH and SH, 40,000 c.p.m. nmol⁻¹ in the case of the mixture). After addition of protein and incubation at about 25 °C for 30 min, 0.58 ml was filtered together with 0.5 pre- and post-washes of FB (filtering buffer, same as BB but lacking BSA and DTT) at a constant rate of 1 ml min⁻¹. The air-dried filters were counted in a scintillation counter. The background level of DNA binding to the filter was 2–3%, and the 100% retention points (indicated on the ordinates) were calculated from aliquots of the DNA stock solution applied directly to the filters. Long headpiece LH (○), short headpiece SH (●), and an approximately equimolar mixture (Δ).

magnetic resonance (NMR) spectroscopy¹⁹ are most likely in the core of the molecule. The accessibility of the amino-terminal tyrosines of native repressor (residues 7, 12, and 17) has also been established in iodination studies²⁰.

The synthetic polynucleotide poly d(A-s⁴T) was selected for further physical measurements because of its unique spectral properties¹⁵. The thioiketo substitution in the position 4 of thymidine produces a strong absorption band in the near ultra-violet (Fig. 2A(b)), well isolated from the protein spectrum, and it also makes possible the efficient photoaffinity labelling of protein-nucleic acid complexes²¹. Poly d(A-s⁴T) forms a helical

structure with Watson-Crick base pairing and features intermediate between the C and D conformations. The pitch height and axial rise are about 10% greater than in the case of poly d(A-s⁴T) (W. Saenger, personal communication). Poly d(A-s⁴T) has been used in studies of *lac* repressor analogous to those described here for the headpiece (T.M.J., in preparation).

The binding of headpiece (LH) to poly d(A-s⁴T) results in altered absorption spectra (Fig. 2B). Hypochromic changes are seen in the s⁴T (360 nm) and, possibly, the dA (260 nm) regions. These cannot be attributed to a disruption of the DNA helix since the latter process leads to a hyperchromic blue shift¹⁵. The sign (positive) and positions of the peaks in the difference spectrum below 300 nm are strongly suggestive of perturbations in the tyrosine spectra, such as from ionisation (or hydrogen-bonding) of the phenolic OH²² or transfer from an aqueous environment to one of higher refractive index²³ (for example, through stacking interactions). If we attribute the change at 286 nm exclusively to tyrosine ionisation and assume that 50% of the headpiece is bound (from data in Fig. 4), then the observed magnitude would be compatible with the complete ionisation of two of the four

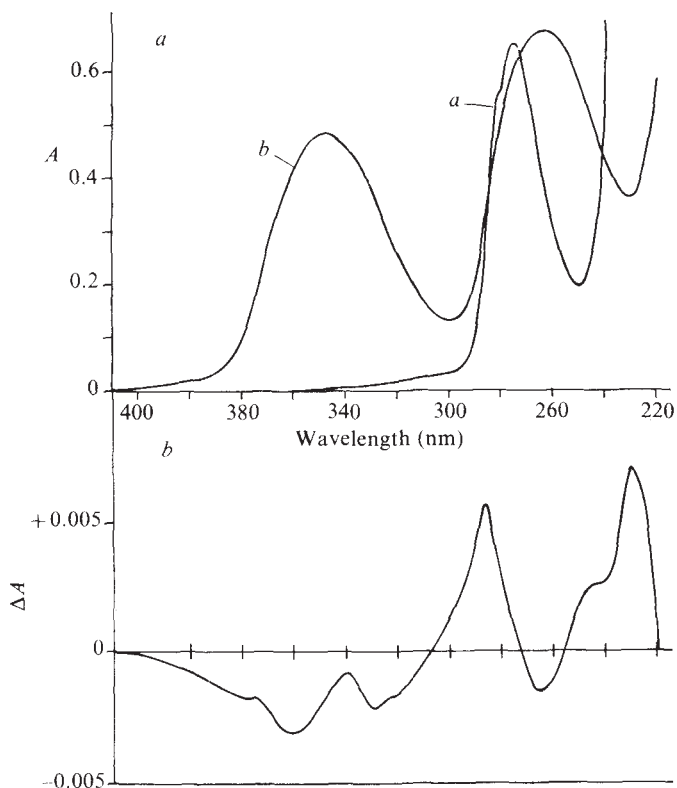


Fig. 2 Absorption and difference absorption spectra of repressor headpiece LH and poly d(A-s⁴T). *A*, Absorption spectra of *a*, 12 μ M LH and *b*, 51 μ M poly d(A-s⁴T) at 20 °C in BB buffer lacking BSA and DMSO. The instrument was a Cary 16. The LH spectrum is 10-fold expanded for clarity. The extinction coefficients for headpiece, based on amino acid analysis, are 4,800 M⁻¹ cm⁻¹ and 5,000 M⁻¹ cm⁻¹ at 280 and 276 nm. ($A_{280}^{1\%} = 7.6$), in agreement with corresponding values for tyrosine. The maxima of the DNA are at 347 and 263 nm and of the protein at 276 nm (minimum at 245 nm). *B*, Difference spectrum between the mixed and unmixed DNA and protein solutions. Tandem cuvettes with 0.438-cm path length in each compartment were used in both sample and reference beams. The solutions shown in (*A*) were used. The difference spectrum was obtained after mixing the sample cuvette. The maxima are at 230 and 286 nm and the minima at 266, 330, and 361 nm. The latter peak corresponds to a 2% decrease in absorbance. The peak at 286 nm represents a 37% or a 4% increase if referred to the protein or DNA contributions alone, respectively. Isoestic points are at 255, 273, and 307 nm. The difference spectrum in this and other experiments was virtually abolished by addition of 0.2–0.4 M KCl. The difference spectrum with the same concentration of SH was similar but 30% lower in magnitude.

Our results are consistent with the above concepts and also relevant to the question of the number of subunits involved. Several studies have established that (at least) two subunits of tetrameric repressor are required for the extremely specific and stable binding to operator^{6,7,38-40}. The interaction of repressor with natural or synthetic non-operator DNA is characterised by a much lower affinity ($K=10^{-4}$ – 10^{-5} M)^{23,40} and an apparent binding length n of 11–16^{27,28,42}. These values are similar to those reported here for headpiece (although we emphasise their tentative nature and can only set an upper limit for n of about 12). The probable correlation between the structure of the headpiece region integrated into the intact repressor protomer and that in the free state after proteolytic cleavage has already been discussed and is further supported by the available data for mutationally-altered

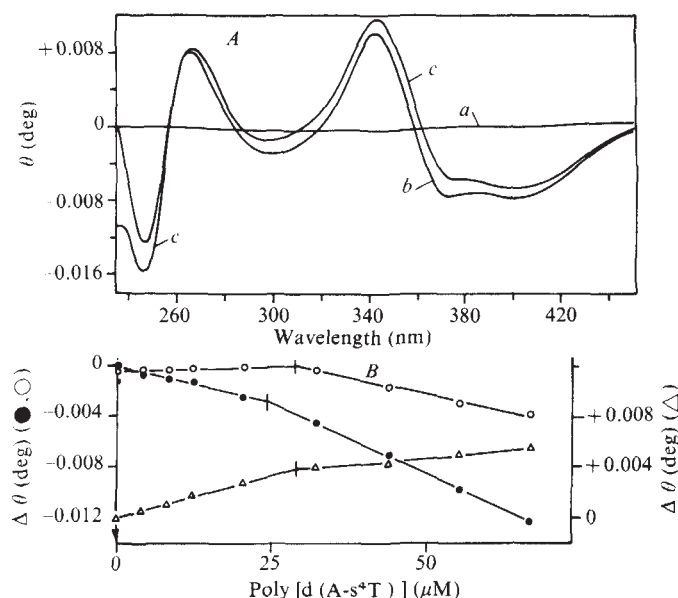


Fig. 3 Circular dichroism spectra of poly d(A-s⁴T) in the absence and presence of headpiece. *A*, CD spectrum of *a*, buffer, as in Fig. 2, at 19.3 °C; *b*, 40 μ M poly d(A-s⁴T), *c*, same solution as in (*b*) plus 26 μ M headpiece. The spectra were measured in selected 1-cm path length absorption cuvettes using the 0.04° range of a Cary 60 equipped with CD attachment. *B*, Titration of headpiece with poly d(A-s⁴T). A 2.3 μ M solution of mixed headpiece in a 1-cm cuvette was placed in the CD spectrometer and measured at 10 discrete wavelengths (only three are shown $\circ$, 300 nm; Δ , 357 nm; $\bullet$, 370 nm). Additions of poly d(A-s⁴T) were made and the cuvette mixed using a pipette but without repositioning. Two linear regions were obtained in the curves at the various wavelengths with intersections denoted by vertical ticks. The data were analysed according to the formalism for ligand lattice interactions of McGhee and von Hippel²⁵. We assume initially the case of a non-co-operative ligand (the protein) for which the binding isotherm is given by the master equation (our nomenclature) $r = L \cdot f(r)/K$ where r is the ratio of bound protein to total lattice sites S_0 (in this case taken as DNA basepairs), K is the intrinsic dissociation constant for an isolated site, L is the free ligand (protein) concentration, and $f(r) = (1 - nr)^n / [1 - (n-1)r]^{n-1}$, a function which takes into account the statistical exclusion of several potential binding sites by the attachment of a single protein molecule covering n consecutive lattice residues. In the initial region of the titration here, the ligand is in excess and the slope is given by $\alpha[1 - (1-\beta)nr]$ where α is the increment in CD signal per unit concentration of free DNA and β is the signal due to complexed DNA relative to that of free DNA residues. From the master equation, r is constant and may approach the value $1/n$ if L/K is large enough (a useful approximation for $r > n/(1+n)$ is given by $r \approx (1/n)[1 - (1-\beta)(K/L)^{1/n}]$). In the second linear region, the lattice is in excess and the slope is equal to α (the observed values are compatible with the spectral changes in (*A*)) and the finite x intercept is given by $\alpha n(1-\beta)L_0$ if the protein is completely bound. It follows that the intersection points defined by the extrapolations of the linear regions obey the relationship $S_0 = L_0/r$. If we assume from Fig. 1 that the LH but not the SH species was quantitatively bound ($L_0(\text{LH}) = L_0/2$, $r = 1/n$), then from these data $n \approx 10$ –14. If these conditions were not fulfilled, n would be lower.

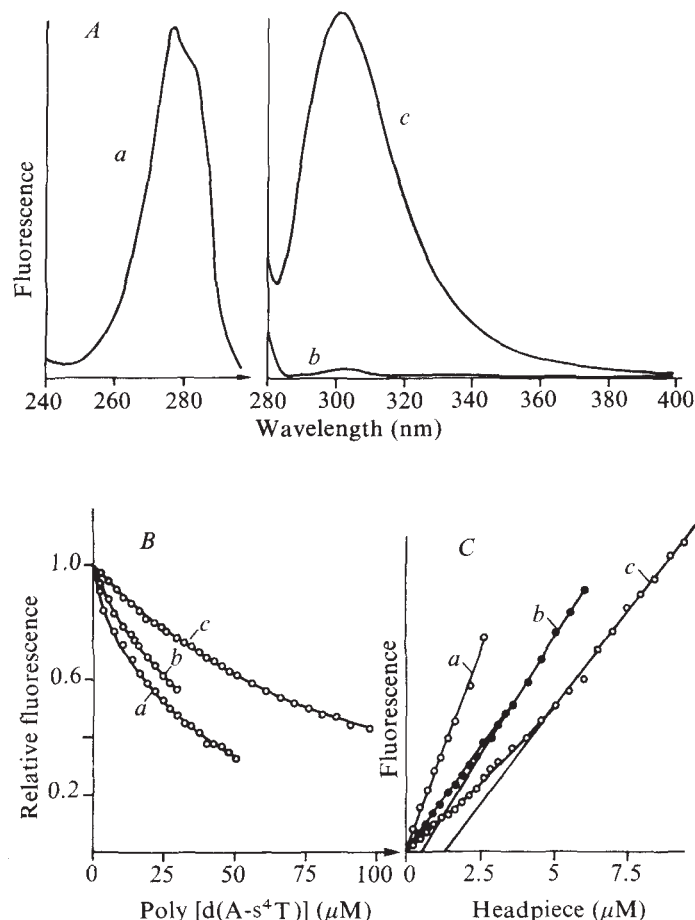


Fig. 4 Fluorescence spectra of headpiece and titration with poly d(A-s⁴T). *A*, Excitation (*a*) and emission (*c*) spectra of 2 μ M LH at 19.5 °C in the buffer given in Fig. 2. A Perkin Elmer MPF4 fluorimeter and 5-mm pathlength cuvettes were used. The spectra were recorded with a 4 nm bandwidth and are uncorrected. The excitation and emission maxima are at 275 and 303 nm, respectively. Curve (*b*) is the buffer blank emission. The spectra are virtually identical with those of tyrosine in the same conditions (the quantum yield of the headpiece, however, has not been measured). *B*, Titration of LH solutions of different concentrations with poly d(A-s⁴T). *a*, 2 μ M LH; *b*, 4 μ M LH; *c*, 13 μ M LH. Excitation was at 275 nm and emission was measured at 303 nm. The emission values after stepwise additions of DNA were corrected for dilution and for inner filter effects due to the DNA absorption (the correction factors were obtained in parallel titrations of tyrosine solutions) and normalised to the corresponding initial intensity. Pronounced curvature is evident and at the highest protein concentration the apparent values of r are improbably high (> 0.23 , corresponding to $n < 4$). In similar experiments with shorter pathlengths and using mixed headpiece preparations (4–7 μ M), the fluorescence quenching was $50 \pm 10\%$ and the apparent stoichiometry 10 ± 2 from the initial slopes. *C*, Titration of poly d(A-s⁴T) solutions of different concentration with LH. *a*, No DNA; *b*, 30 μ M poly d(A-s⁴T); *c*, 48 μ M poly d(A-s⁴T). The titrations were performed in 7-mm path length cuvettes mounted in a temperature-jump kinetic fluorimeter²⁹ using phase-sensitive detection. Excitation was at 280 nm and the emission above 305 nm was collected. Several additions of concentrated headpiece were made from a microsyringe and mixed with a magnetic bar. In contradistinction to the experiments in (*B*), the initial phases in these titrations are characterised by excess lattice (DNA) and thus protein–protein interactions are minimised. Ultimately, headpiece is in excess and the final linear slopes result from the addition of free protein (they are not identical in the three curves due to the different, but constant, inner filter effects caused by the DNA). The ratio γ of the initial to the final linear slopes of each curve obeys the relationship $1/(1-\gamma) = (K/S_0 + 1)/(1-\beta)$ from which K (the dissociation constant for an isolated complex) has a value $> 10 \mu$ M and β (the relative fluorescence of the complex) ≈ 0.5 . The x intercepts of curves (*b*) and (*c*) are given by $S_0(1-\beta)/n$, assuming saturation of the DNA, from which $n \approx 12$. The true value may be lower, however, since saturation will not have been achieved unless co-operative binding at the higher protein concentration occurs and is characterised by a much lower value of K .

molecules (Table 1). The results open the possibility that the nonspecific DNA binding of native repressor in certain circumstances may involve only a single subunit of the tetramer.

We now re-examine the apparent co-operativity in headpiece binding to DNA as seen in the filter assay (Fig. 1*b*). This phenomenon could arise from (1) a concentration-dependent aggregation of headpiece to form a species with higher affinity for the filter and/or DNA; (2) a requirement for several bound protein molecules per DNA chain for successful fixation to the filter, either because of weak interactions with the latter or rapid dissociation of the binary or tertiary complexes; and (3) true thermodynamic co-operativity, that is, strong positive interaction between adjacent bound protein molecules. The chromatographic characterisation of isolated headpiece provides no evidence for (1)⁴⁰. Some possibilities under (2) are excluded by the stability of the filter-bound complex to moderate washing and the absence of a strong

chain length dependence. Certain indications for co-operativity (3) exist and are discussed in the figure legends. It has not been possible to check for sigmoidicity in solution studies by measuring a property of the DNA during addition of protein. The signals are simply too small (Figs 2, 3). Yet the behaviour at low concentration of the headpiece derived from the BG2 mutant repressor (Table 1) is most easily understood in terms of reduced co-operativity and thus a more uniform distribution of bound molecules. Retention by the filter may also require 'clusters' of headpiece or a given threshold level of complex formation. It is possible that the low values of n seen at high protein DNA ratios (Fig. 4B) result from overlapping between binding sites, that is, occupancy by more than one headpiece molecule of the same stretch of duplex DNA. Electron microscopic evidence has been obtained recently for such a phenomenon in the case of native repressor bound nonspecifically to DNA at low ionic strength^{4,3}.

The system described here is ideal for probing general features of protein-nucleic acid interaction due to its inherent properties and the potential for extensive biochemical⁷ and genetic^{5,9} manipulations. Further physical studies using dynamic luminescence and high resolution NMR techniques are in progress.

We thank Dr B. Müller-Hill for supplying some of the bacterial strains, and Maria van der Ploeg for technical assistance.

Received 25 March; accepted 10 August 1977.

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letters to nature

Electron scattering in X ray-emitting galaxies

SEVERAL galactic nuclei have been identified as luminous sources of X rays. These include the radio galaxy Cen A (refs 1 and 2), several type I Seyfert galaxies^{1,3-5} such as NGC 4151, 3C120 and 3C390.3. The quasar 3C273 has also been detected^{1,3}. Most of these sources have been observed to vary and have, therefore, been considered compact. Their X-ray spectra, where measured, seem to indicate significant low energy absorption intrinsic to that galaxy. Examples are Cen A (ref. 2) for which the line of sight hydrogen column density (assuming solar abundances) $N_H \sim 10^{23} \text{ cm}^{-2}$ and NGC4151 (ref. 6) where $N_H \sim 5 \times 10^{22} \text{ cm}^{-2}$.

The optical depth to electron scattering, τ_{es} , is unity when $N_H \sim 1.5 \times 10^{24} \text{ cm}^{-2}$. Consequently τ_{es} is ~ 10 and 5% for Cen A and NGC4151 respectively. Higher values of τ_{es} may apply if the heavy elements are underabundant, or if a significant fraction of them is completely ionised. A scattered flux of X rays is thus to be expected from such galaxies. This flux may be detected in the time variations of the compact source if the bulk of the scattering material lies in the nucleus. A fraction $\sim \tau_{es}$ of the variations are broadened by the light travel time across the scattering region. It is likely, however, that much of the gas lies in the body, or disk, of that galaxy. In this case the scattered flux may be spatially resolvable, appearing as a diffuse extended source surrounding the nucleus. The total intensity of this extended source, I_{es} , is $\sim \tau_{es}$ times the mean intensity of the compact source, I_c (provided that $\tau_{es} \lesssim 1$). τ_{es} is the electron scattering optical depth weighted as to

luminosity and averaged over all directions. Note that it need not be equal to τ_{es} deduced from X-ray absorption measurements.

The spatial structure of the scattered flux depends on the underlying gas distribution and the past activity of the compact source over a time equal to that taken by light to cross that region ($\lesssim 10^4$ yr). The variability of the compact source implies that its observed intensity, I_c , need not equal $\bar{I}_c$, and thus I_{es} need not equal $\bar{\tau}_{es} I_c$. I_{es} will, of course, exceed $\bar{\tau}_{es} I_c$ in approximately half of any sample of galaxies. It is in principle possible that the past X-ray history of the nucleus can be determined by comparing independent measurements of the gas density (for example, 21 cm if the gas is predominantly neutral) with the spatial structure of the extended source.

The spectrum of the scattered X rays should be similar to that of the compact source above ~ 8 keV, but a low energy cut off due to photoelectric absorption at energy E_a should occur at $E_a \approx 8 \bar{\tau}_{es}$ keV. This cutoff is related to the observed cutoff, E_{ao} , by

$$E_a \approx \left(\frac{\bar{\tau}_{es}}{\tau_{es}} \right)^{1/3} E_{ao}$$

This may allow $\bar{\tau}_{es}$ to be determined. Grazing-incidence telescopes such as are to be carried on HEAO-B are ineffective above ~ 4 keV, and may only detect the innermost scattered flux. Modulation collimators (such as on HEAO-A), or the lunar occultation technique (for example, EXOSAT), may prove more useful in mapping the complete scattering region. Photoelectric absorption by iron results in an absorption edge

and fluorescence emission line in the observed spectrum. The equivalent width of this line (in the scattered spectrum) is ~ 900 eV, assuming solar abundances. The scattered X rays should be highly polarised, the degree of polarisation being maximum ($\sim 100\%$) perpendicular to an edge on disk.

Both Cen A and NGC4151 are good candidates for the detection of scattered X rays. The scattered flux may already have been detected in the case of Cen A, for Delvaile⁷ reports the detection of a 3 arc min diameter X-ray source coincident with the compact source. Cen A bears some resemblance to an edge-on spiral⁸, and might therefore be a candidate for polarisation studies. The disk of NGC4151 is observed nearly face on^{9,10}, with a mean neutral hydrogen surface density over the optical spiral structure (8 arc min diameter) of $\sim 7 \times 10^{-4}$ g cm⁻² (ref. 8). τ_{es} perpendicular to the plane of the disk is thus $\sim 5 \times 10^{-4}$, which is $\sim \tau_{\text{es}}$ for the case of a thin disk containing a central source radiating isotropically. This indicates a very weak scattered flux, I_s , from most of the disk, if $I_c \sim \bar{I}_c$. τ_{es} and thus I_s may be considerably greater within the central few kpc, especially if much of the reported low energy absorption⁶ of the presumed compact source occurs in this region. Note that this compact central X-ray source may maintain a considerable fraction of the lightest elements in the inner parts of NGC4151 in an ionised state. The electron scattered flux in the infrared, optical and ultraviolet wavebands should be reddened in a similar manner to the direct flux that is detected from the galactic centre. This assumes that NGC4151 contains a distribution of dust similar to that in our Galaxy. The continuous optical spectrum of the bar in NGC4151 does, however, more closely resemble that of its nucleus than that expected from blue stars¹⁰. Optical polarisation studies could rule out electron scattering, which would imply a high degree of polarisation perpendicular to the bar.

Finally, it is worth noting that the extended X-ray source at the galactic centre¹¹ may in part be due to scattering of the X rays from the strong sources in that region.

I thank J. E. Pringle and M. J. Rees for helpful discussions.

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Received 25 July; accepted 30 August 1977.

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Absorption lines in the optical spectrum of quasar AO0827+24

In January 1977 the radio source AO0827+24 (ref. 1) was observed at 90 GHz by F. Owen, S. Mufson, R. Porcas and T. Moffett with the NRAO 36-foot antenna at Kitt Peak, during observations of several flat spectrum sources selected from the Green Bank 5-GHz survey². Among these sources, 34, including AO0827+24, were found to have flux densities greater than 1 Jy at 90 GHz. Spectrographic observations of the optical counterparts of these 34 sources are in progress. We report here observations of the Mg(II) and Fe(II) absorption line system, at a redshift $z = 0.525$, in the optical spectrum of the 17.5-mag stellar object associated with AO0827+24 because of the similarity with the absorption line system of the two BL Lacertae objects PKS0735+178 (ref. 3) and AO0235+164 (ref. 4) which also have flat radio spectra.

We obtained trailed image tube spectra of AO0827+24 with a moonlight eliminator, using the 2.1-m and 2.7-m telescopes at McDonald Observatory in February and March 1977. We first took two spectra at 230 Å mm^{-1} covering the wavelength range 3,500–6,200 Å. These spectra showed a low contrast feature in emission near 3,700 Å. We later realised that a line at this wavelength had been seen in previous observations of AO0827+24 (refs 5 and 6). Wills and Wills⁶ propose an emission line redshift of 2.046 based on the line at 3,700 Å being identified with $\text{L}\alpha$ and another emission line which they observe at 4,063 Å being identified with $\text{O(II)}\lambda 1335$; they also note that against this interpretation is the apparent absence of $\text{C(IV)}\lambda 1549$ and that the emission line redshift of this object is still in doubt.

The most conspicuous feature on our low-dispersion spectra was an absorption line at 4,270 Å. We therefore obtained spectra with a higher dispersion, 103 Å mm^{-1} , in the range 3,500–5,400 Å. On the best spectrum the feature at 4,270 Å is clearly resolved into two components at 4,262.5 and 4,273.5 Å and another absorption line is observed at 3,966.8 Å.

The identification of the doublet with Mg(II) 2,795.53, 2,802.70 Å gives a redshift of 0.5247. Using this value the line at 3,966 Å corresponds to a rest wavelength of 2,601.7 Å and is identified with Fe(II) 2,599.40. This line may be contaminated by the interstellar line Ca(II) 3,968 but is probably not caused solely by it otherwise we would expect to see Ca(II) 3,933 with a comparable strength, and this is not observed on our spectra.

Since the Mg(II) doublet is resolved, the full line width in the rest frame of the object is less than 7 Å or 750 km s^{-1} . The real width of the redshifted lines is not known and may be much smaller than the resolution of our spectra, which is about 8 Å. Clearly the absorption line spectrum of AO0827+24 deserves further observation, however, the available data suggest that the absorption spectrum of AO0827+24 has marked similarities with that of PKS0735+17 and AO0235+16. Since there is at least one emission line in the spectrum of 0827+24, the object is not a true BL Lacertae object. But recent results indicate that the spectrographic differences between BL Lacertae objects and quasars are not as clear cut as previously thought⁷.

In PKS0735+17 (ref. 3) only the Mg(II) doublet has been so far detected and gives a redshift $z = 0.424$. In AO0235+16 (ref. 4), there are two redshift systems, one at $z = 0.851$ determined from the Mg(II) doublet, the other one at $z = 0.524$ (which is strikingly close to the redshift of AO0827+24) determined from the Mg(II) doublet and lines of Mg(I), Fe(II) and Mn.

Interestingly, the four absorption-line redshifts in the optical spectra of these objects are all between 0.4 and 0.85. This situation may be caused in part by selection effects inherent to the spectrographic observations: the range of redshift 0.4 to 0.85, corresponds to the easily recognisable Mg(II) doublet falling between 3,900 and 5,200 Å where the search for absorption lines is the easiest.

The sample mentioned above, comprising 34 sources stronger than 1 Jy at 90 GHz, includes AO0827+24 and also the two BL Lacertae objects PK20735+17 and AO0235+16. Thus at least three out of 34, or about 10% of the objects, show similar absorption line systems with $z < 0.9$; the incidence of such absorption systems is therefore not uncommon. It is not known at present whether this phenomenon is caused by cold gas clouds ejected by the continuum emitting source or by intervening galaxies. If these absorption lines are caused by intervening galaxies, they should be present in the same percentage of the spectra of all QSOs with $z > 0.9$ regardless of their optical and radio properties. The incidence of absorption lines in the spectra of QSOs with emission lines of width and strength similar to those found in BL Lacertae objects is not known since up to now QSOs have generally been observed with a low dispersion sufficient to detect broad emission lines but inadequate to resolve and thus recognise the Mg(II) doublet; this is in contrast to the spectra of the BL Lacertae objects, especially the bright ones, which are thoroughly searched for

absorption lines. Clearly a systematic search for absorption lines in the spectra of QSOs will be useful for determining their origin.

NRAO is operated by Associated Universities, Inc., under contract with the NSF.

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Prebiotic polymers and infrared spectra of galactic sources

INFRARED absorption features characteristic of molecular dust clouds in the Galaxy may be assigned to complex organic polymers or prebiotic polymers. It could be argued that such highly stable, complex polymers evolve due to radiation processing of molecular mantles on interstellar grains—essentially by a type of natural selection which operates in the interstellar medium. A large fraction of all C, N, O elements in the interstellar medium could be condensed in the form of these stable polymers. Such interstellar material may also account for a significant fraction of the 'insoluble organic matter' which is found in carbonaceous chondrites.

The chemical composition of interstellar dust is uncertain. Observations of infrared excesses in cool stars combined with appropriate thermodynamic calculations support the view that grains comprised of silicates, iron and graphite are produced in these stars¹. It is unlikely, however, that these materials alone can satisfactorily explain the entire range of optical data relating to interstellar grains². Measurements of interstellar linear and circular polarisation point strongly to the dominance of a dielectric grain material at optical wavelengths³. Furthermore, estimates of the mean extinction coefficient of interstellar matter together with cosmic abundance data imply that the large bulk of grain material is comprised of C, N, O elements². These elements, in appropriate combinations with hydrogen, may exist as molecular mantles on grains. Mixtures of inorganic ices were proposed by van de Hulst⁴;

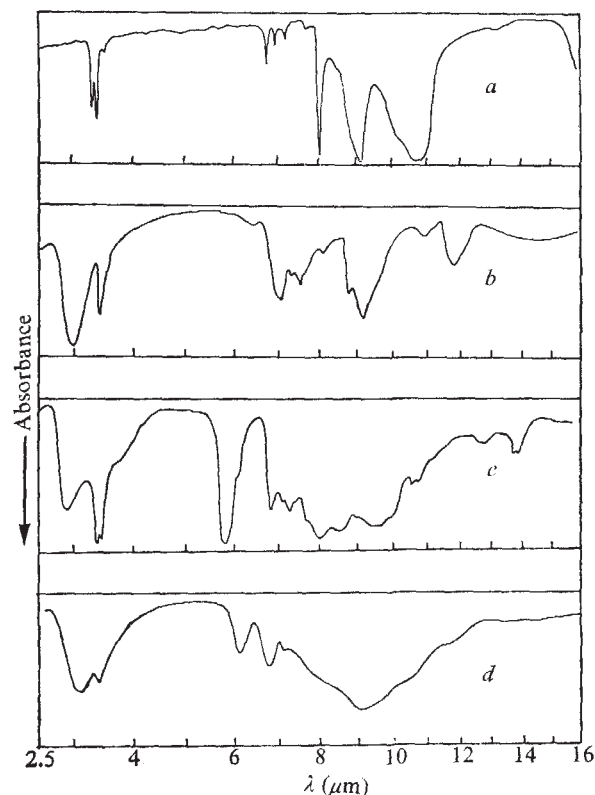


Fig. 2 Absorbance (A) of organic polymers as a function of wavelength. *a*, Polyformaldehyde (Delrin acetal resin); *b*, polyvinyl alcohol; *c*, natural resin: Shellac—regular bone dry, bleached—FEXO type¹². *d*, Average absorbance for 18 selected polymers of various types.

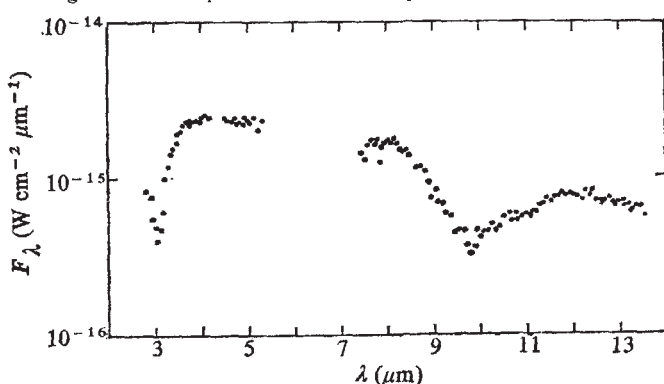
and N.C.W.⁵ has discussed the possibility of formaldehyde polymers. These two types of composition could represent intermediate stages in a progression towards an increasingly complex macromolecular structure for grain mantles.

We envisage the evolution of grain mantles in two distinct steps. First a mixture of relatively simple organic and inorganic ices (for example, CO, H₂O, NH₃, H₂CO) condenses on silicate grains in interstellar clouds. The combined effect of interstellar cosmic rays, X rays and ultraviolet photons on these grains will be to initiate solid-state polymerisation reactions of the type discussed by Goldanskii⁶. The end product of such processing of grain mantles is a stable, radiation-resistant organic polymer or prebiotic polymer. Such polymers may be thought of as evolving according to a type of natural selection in response to a wide range of selective pressures operative in the interstellar medium. It is possible that the low abundances of simple molecules and radicals (for example, OH, CH, CN) observed in diffuse interstellar clouds is maintained by a very slow degradation of these polymers.

Infrared absorption features observed in galactic sources, include bands centred on 3.1, 3.4 and 8–12 and ~20 μm. The most extensive observational data are available for the 8–12 μm feature. This band is seen in emission by hot dust in HII regions and circumstellar shells, as well as in absorption against sources of continuum infrared radiation. A silicate explanation for the 10 μm band seems natural for many sources exhibiting this feature, particularly in cases where dust is observed in circumstellar shells around oxygen-rich M giant stars⁷. Similarly, an H₂O-ice explanation for the 3.1-μm band may be tenable in some cases. In sources of exceptionally strong absorption, for example, the BN object in the Orion nebula, there are inadequacies in this conventional point of view.

Figure 1 shows the infrared spectrum of the BN object, which is probably one of the most heavily reddened objects in the Galaxy⁸. Here we see absorption bands centred on wavelengths close to 3

Fig. 1 Infrared spectrum on the BN object in the Orion Nebula⁸.



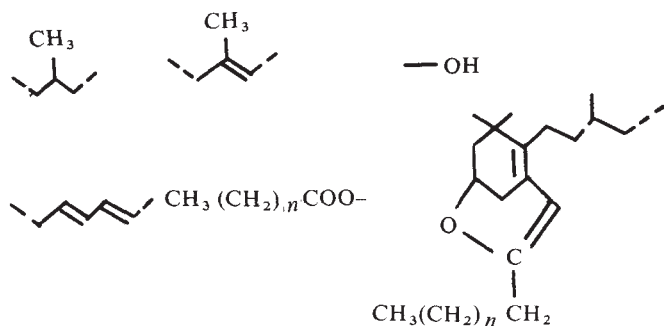
and 10 μm . The 10- μm band is usually attributed to silicates, and the 3- μm bands to H_2O -ice. Both these compositional assignments present problems. If we assume that the overall composition of dust which obscures the BN object is representative of grains in the general interstellar medium, the mass ratio of ice to silicates which is consistent with the observed relative strengths of the two bands in Fig. 1 is inconsistent with cosmic abundance criteria^{2,9}. Also, the detailed shape and width of this 10 μm band does not give a satisfactory fit with the absorption spectrum of any known silicate.

Table 1 Vibrational frequencies for various linkages in organic molecules¹¹

Bond	Wavelengths (μm)
C-C, C-O, C-N	7.7-12.5
C=C, C=O, C=N, N=O	5.3-6.7
C $\equiv$ C, C $\equiv$ N	4.4-5.0
C-H, O-H, N-H	2.6-3.7

Another case which presents difficulties for simple silicate-ice models is the infrared spectrum of a source at the galactic centre. A broad 3- μm band, which includes a weak feature at 3.4 μm , cannot be explained in terms of a simple model of H_2O -ice grains¹⁰. Likewise, the deep, narrow absorption band centred on 9.7 μm in this source⁷ cannot readily be explained by silicate-type grains.

The possibility that organic polymers could contribute to absorptions at 3, 3.4 μm , and over the 8-12- and 18-24- μm wavebands in galactic sources suggests that organic polymers could provide a potentially more flexible and viable explanation of astronomical absorption spectra at near and mid-infrared wavelengths compared with silicate grains. The mass density of absorbing material in this former category may be higher by an order of magnitude, because of the higher cosmic abundance of C, N, O elements.

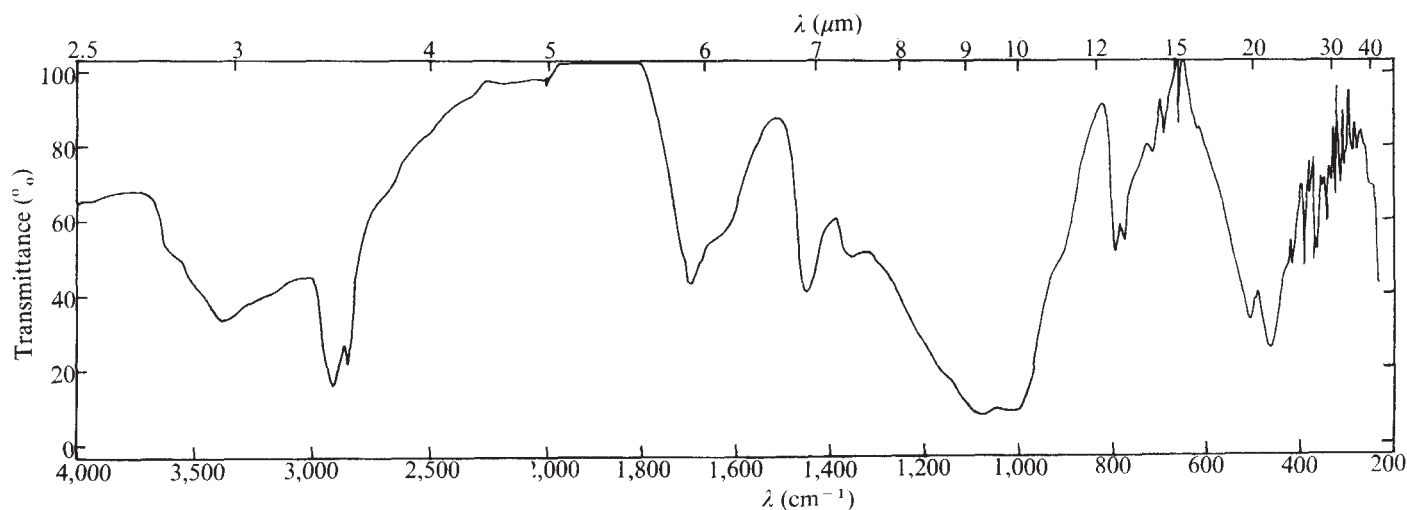


Organic molecules and polymers are known to have broad absorption bands in the wavelength region 1-100 μm due to transitions between vibrational-rotational levels. Over the waveband 2.5-13 μm considered above, stretching modes of elementary bonds which contribute to absorptions are listed in Table 1.

The precise positions of band centres and band widths appropriate to individual groups depend on associated linkages and on the particular molecular environment in which these groups are placed. From extensive spectral data¹² on organic polymers, absorption features in the wavelength ranges 8-12 and 2.5-3.5 μm are seen to be the most persistent properties encompassing natural and synthetic polymeric compounds of widely ranging types.

Figure 2 shows the absorbance spectra of an acetal resin (a), polyvinyl alcohol (b) and a natural resin (c). While we could not find a single spectrum of a simple polymer which provides a satisfactory fit to the data in Fig. 1, the average spectrum of a number of different types of polymer tends to produce a relatively structureless, broad 10- μm band giving better agreement with this astronomical absorption feature. Figure 2d represents a synthetic spectrum obtained simply by averaging absorbance data^{11,12} for a selection of 18 polymeric species. The selection was made so as to include polymers of a wide variety of types, but with a bias towards those which exhibited broad bands centred near 3 μm due to either O-H or N-H stretching modes. This 'synthetic' curve is fairly close to a smoothed out mean of the three absorbance spectra Fig. 2a, b and c. Although we cannot claim any uniqueness for our synthetic spectrum (d), this curve provides significantly better agreement with the BN source data (Fig. 1) than any model involving silicate-ice grains. This cannot be dismissed as fortuitous. A smooth absorption feature of appreciable strength in the 8-12 μm waveband arises quite naturally for an average polymer spectrum, if we take a large enough number of polymers of various types^{11,12}. But the requirement of either a strong 3- μm band or a 3.4- μm band demands a more restrictive choice. In this context it is worth noting that some galactic sources show strong bands at 3.4 μm , but not the usual 8-12- μm feature. A case in point is the emission spectrum of the planetary nebula NGC7027 (ref. 13). It is also interesting that some astronomical data, including the extended infrared emission source in the Trapezium nebula¹⁴, indicate a significant absorption coefficient in the '10- μm band' of interstellar dust extending to $\lambda \geq 13 \mu\text{m}$. There are other cases where a 3- μm absorption band, wider than one which can usually be attributed to ices, occurs together with a broad 8-13- μm band¹⁵. Such absorptions cannot readily be explained by silicates and H_2O -ice but could be obtained by biasing our organic compound mix to include a substantial fraction of amides. These compounds have a broad 3- μm feature due to N-H stretching as well as a broad absorption feature in the 12.5-15- μm region due to out-of-plane NH wagging¹¹; a typical case is the spectrum of isobutyramide¹¹.

Fig. 3 Infrared absorption spectrum of sporopollenin from *Tasmanites punctatus* fossil planktonic algae. Sporopollenin is an oxidative co-polymer of carotenoid-carotenoid esters containing the molecular and macromolecular groupings shown above.



An exceedingly complex organic or prebiotic polymer which evolves in the interstellar medium could well possess absorption properties similar to those of the synthetic polymer spectrum Fig. 2d. Alternatively, the average interstellar absorption curve may be due to an ensemble of less complex polymers which has an average spectrum similar to Fig. 2d. In the context of the former possibility, it may be of interest to consider the properties of highly stable naturally occurring biopolymers.

A particularly stable polymer of this type is sporopollenin, an oxidative copolymer of carotenoid-carotenoid esters, which forms a major component of pollen and many spore walls. Figure 3 shows the infrared absorption spectrum of sporopollenin from *Tasmanites* fossil planktonic algae over the wavelength region 2.5 to 40 μm . This spectrum shows a broad absorption feature at 3 μm , a narrower 3.4- μm band, and a deep and somewhat narrow absorption band at 9.7 μm generally similar to data for the galactic centre infrared source^{7,10}. In Fig. 3 there is also a band at 20 μm similar to one which is regarded as providing additional evidence for interstellar silicates. Sources where the detection of a 20- μm band has been claimed include the Trapezium and KL nebulae¹⁶.

Although these spectral similarities do not prove the presence of sporopollenin in interstellar space, nor indeed of any biopolymer, there is an indication here of a fairly complex, radiation-resistant prebiological polymer associated with interstellar grains. It is possible that the conversion of interstellar grain mantles into radiation resistant polymers, and the formation of protective coatings on pollens and spores followed similar chemical pathways, in response to broadly similar selective pressures. Prebiotic polymers with large-scale molecular groupings similar to those occurring in sporopollenin (but less complex) may be the net result of such an evolution. Infrared spectra of these two systems could be fairly similar.

We have already suggested an identification of the λ 2,200 Å interstellar absorption band with electronic transitions in conjugated multiple bonds of prebiotic molecules¹⁷—such molecules being supposedly trapped within polymeric matrices of the type considered here. Sakata *et al.*¹⁸ have also shown that a similar ultraviolet absorption feature occurs in the soluble organic matter extracted from a carbonaceous chondrite. This similarity in ultraviolet spectra was interpreted as support for the inclusion of primitive grain clump material in meteorites. In view of the infrared spectral identification suggested here, we might further argue that the insoluble organic matter of carbonaceous chondrites may also have an interstellar origin. This material is too complex for detailed identification, but is believed to be comprised of highly condensed aromatic polymers which account for nearly 70% of the total carbon content in these meteorites¹⁹. Such prebiological polymers, processed in the interstellar medium, could have played a crucial part in the evolution of terrestrial life.²¹

We (F.H. and N.C.W.)²⁰ have also shown a strong case for the class of biopolymer—polysaccharides—in interstellar dust. Other organic polymers such as discussed above would probably be less abundant than polysaccharides.

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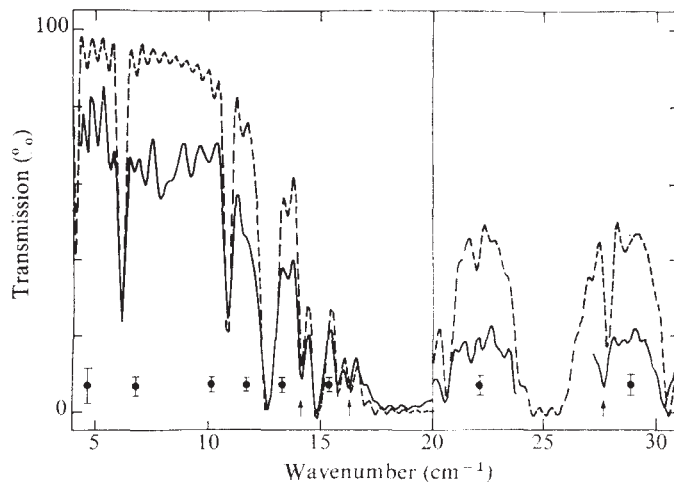
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Atmospheric absorption between 4 and 30 cm^{-1} measured above Mauna Kea

CONSIDERABLE attention is being given to the construction of new telescopes which will extend ground-based astronomical observations to millimetre and sub-millimetre wavelengths, and so it is essential to have a better understanding of atmospheric absorption at these wavelengths. We report here measurements made from the summit of Mauna Kea in Hawaii of the absorption of radiation coming through the atmosphere in slant paths from the sun. These have extended previous knowledge of absorption at this site^{1,2} to lower frequencies and have confirmed that a little understood phenomenon which we call anomalous absorption can greatly reduce atmospheric transparency at some millimetre wavelengths theoretically predicted to be good windows. To compare our measurements with theoretical predictions, particular attention was paid to determinations of atmospheric water content in the same path from the sun. A complete absorption band near 1.4 μm was scanned, therefore, using a monochromator and values of precipitable water were derived by well known formulae³.

Spectra in the wavenumber range 4–30 cm^{-1} were obtained by Fourier inversion of the output of a Michelson interferometer which was illuminated by a 50-cm diameter telescope and used a Golay detector. Interferograms giving a resolution of 0.5 cm^{-1} were recorded in 30 min. As the sun is nearly a black body at these frequencies, a local black-body source was used

Fig. 1 Measurements in January–February 1976 at Mauna Kea (elevation 4.2 km, latitude 19°50', longitude 155°28'). The solid line is an average transmission spectrum for which the mean zenith angle is 55°. The resolution is 0.5 cm^{-1} . Below 20 cm^{-1} the average is from nine days of observation with a mean water vapour content of 1.9 mm precipitable in the path. Above 20 cm^{-1} the average is from the four driest days, with 0.9 mm precipitable. The displaced error bars represent the standard deviation of the noise on the spectra. The dotted line shows theoretical predictions in which the small oscillations between known lines come from including the effects of the instrumental 'spectral windows'. Arrows mark O_2 lines, other lines are due to water.



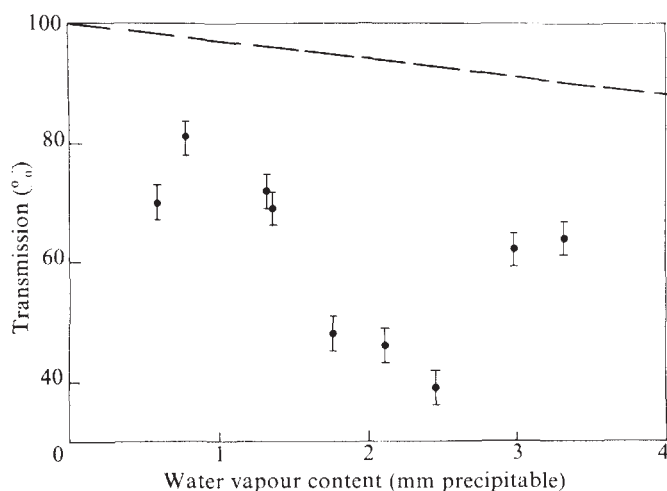


Fig. 2 Daily averages of transmission at 7.7 cm^{-1} plotted against the measured water vapour content. Mean zenith angles are in the range $47\text{--}61^\circ$. The dashed line shows predicted transmission.

Kea. The observed magnitudes are similar to values previously obtained at Mauna Kea¹, and other mountain sites^{6,7}. The variability in the effect which we observed is sufficiently great that these observations are not necessarily in conflict with the low values recorded at White Mountain, California⁸. The amount of anomalous absorption in the range $7\text{--}9.5\text{ cm}^{-1}$ is consistent with what has been found in horizontal atmospheric paths⁹ if reasonable values of scale height for absorbers are assumed. The non-zero intercept on the absorption against

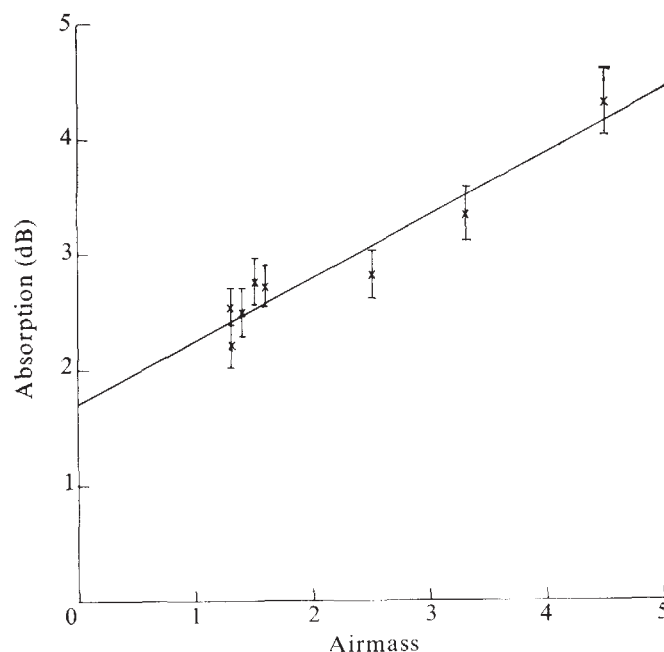


Fig. 3 Each point, which represents absorption averaged over the 7.0 cm^{-1} to 9.5 cm^{-1} interval, was measured in the same day. The error bars give an estimate of the standard deviation of the noise in this interval. Airmass is defined as the ratio of the amount of atmosphere along the observing path to the amount in the zenith direction. The line fitted to the data gives an intercept of $1.7 \pm 0.2\text{ dB}$.

to provide a calibration by which day-to-day changes in radiometric sensitivity could be followed and also give a reference spectrum by which the solar spectra were divided to measure atmospheric transmission. As we could not record a solar signal without absorption, measurements for one day of particularly high atmospheric transparency were used to establish the transmission scale. We selected a wavenumber of 10.3 cm^{-1} where there is low atmospheric absorption and assumed that the only loss was that predicted by the theoretical model corresponding to the measured water content. We found that on that day the signal strength varied with zenith angle by the expected amount. Figure 1 shows a composite spectrum from several observations averaged as described in the legend. These data were obtained in clear conditions and for the wavelengths under discussion this means that attenuation by particles could be neglected. Theoretical spectra were calculated from AFRL tabulations of molecular line parameters⁴.

As well as the expected lines of water and oxygen, additional absorption was observed which seriously affects 'windows' used for astronomical observation. The extra component is described as anomalous absorption and no simple correlation between its strength and atmospheric water content was found. Figure 2 shows this in the behaviour of the atmospheric window near the astronomically important $J = 1 \rightarrow 2$ pure rotation line of carbon monoxide at 7.7 cm^{-1} . At this frequency the average absorption was 2.1 dB where the water vapour and oxygen model predicts 0.23 dB for the mean water content of 1.9 mm precipitable.

As an alternative to radiometric calibration, it is common practice to assess atmospheric absorption by measuring signals as a function of the zenith angle from an astronomical source. Assuming that the absorbers are horizontally stratified and do not vary in concentration during a sequence of measurements, a plot of absorption against airmass would be a straight line through the origin. Plotting absorption values for the interval 7 cm^{-1} to 9.5 cm^{-1} measured in this way, we found that the intercept at zero airmass was significantly greater than zero on most days, as shown in Fig. 3. This suggests that stratification of the molecules giving rise to anomalous absorption may be affected by the presence of the mountain and that the method of calibration based on zenith angle dependence, though widely used, may not apply in such circumstances.

We conclude that anomalous absorption, frequently reported⁹ in qualitative terms, is a major phenomenon at Mauna

airmass plot suggests that there may be factors specific to a site which can enhance anomalous absorption.

We thank Professor J. T. Jefferies for the use of Mauna Kea Observatory, University of Hawaii, also Mr T. Krieger and his staff at the Observatory for their assistance.

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Received 27 April; accepted 30 August 1977.

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Carbonate mineral detection by variable atmosphere differential thermal analysis

GREATLY improved detection limits for the presence of anhydrous carbonate minerals in mixtures with other types of minerals can be obtained by variable atmosphere differential thermal analysis (DTA) using dynamic furnace atmospheres of CO_2 (flow rate 100 ml min^{-1})¹. Details will be published elsewhere^{2,3} (see ref. 1 for details of the Du Pont unit and conditions of analysis). These carbonates fall into two groups; those which decompose in CO_2 with single, or multiple⁴ endothermic dissociation reactions liberating CO_2 . The first group contains, calcite CaCO_3 , magnesite MgCO_3 , smithsonite ZnCO_3 , and siderite FeCO_3 (ref. 5); while the second includes dolomite $\text{CaMg}(\text{CO}_3)_2$, ankerite $\text{Ca}(\text{MgFe})(\text{CO}_3)_2$ and cerussite PbCO_3 (ref. 4). Here calcite and dolomite are taken as typical examples and their behaviour described.

In conditions of dynamic CO_2 compared with static air, the single endothermic peaks of group one members show several important DTA curve modifications (Fig. 1*b* and *c*): peaks become narrower, and more sharply defined with much increased peak heights; the complete peaks, including initial, peak and final temperatures, move up scale to occur at considerably higher temperatures.

The multiple endothermic peaked second group behave differently in the same conditions (Fig. 1*d* and *f*): the initial peak becomes displaced down scale^{2,6} (sometimes, due to crystallinity and equipment, for low concentrations, the known fall in peak temperatures due to dilution when determined in air⁶ and their relative stability in dynamic CO_2 may cause both dolomite peaks to appear up scale from the fused composite peak representing them in air (Fig. 1*d* and *f*)). The higher temperature peak (dolomite) or peaks (ankerite) move up scale to occur at higher temperatures with considerably increased and decreased peak heights and widths respectively; markedly increased peak sep-

aration and definition results. Individual peaks remain recognisable down to the limits of detection (see Fig. 1*e*) and do not, with progressive content dilution coalesce into the single broad similar (difficult to identify) features which typically result from determinations in air (compare Fig. 1*d* and *f*). The size of the now clearly resolved middle endothermic peak of ankerite, varies with iron content².

The DTA of such carbonates in these conditions enables their greatly improved detection down to contents in the order of 0.25%³, the identification of members of the dolomite-ferroan dolomite-ankerite series² and the detection of 'iron carbonate' components, present either as siderite or ankerite-ferroan dolomite with detection limits and content evaluations² which seem considerably superior to those obtainable by routinely available X-ray diffraction data, which emphasises the potential of DTA in this aspect of determinative mineralogy.

I thank the University of Bristol (Geology) and the Macaulay Institute for Soil Research (Pedology) for the opportunity to undertake this work during the tenure of a visiting professorship.

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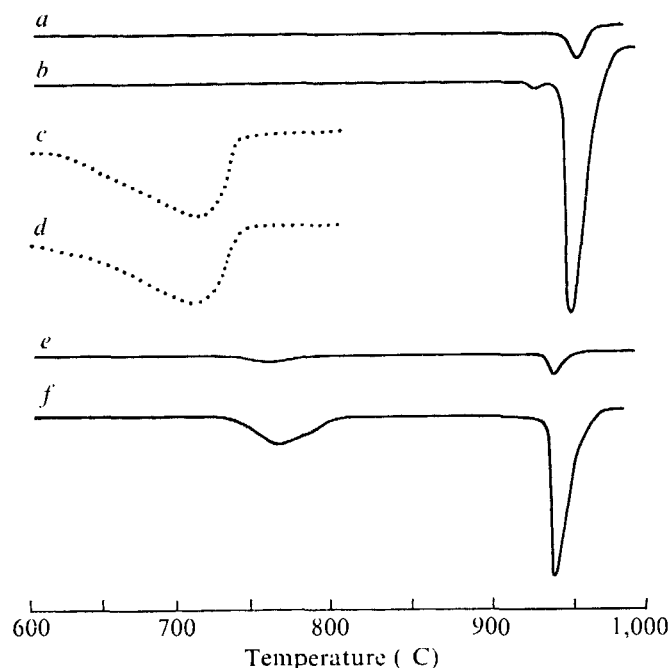
Δ^2 -Sterenes as diagenetic intermediates in sediments

A SMALL fraction of the sterols derived from living organisms is found intact or partially degraded in the geological environment. Seawater and many recent or relatively immature older sediments contain unaltered, or only slightly altered sterols¹⁻⁸, whereas in older sediments and petroleum these compounds have usually been transformed by redox reactions into their saturated (steranes) or partially aromatised counterparts⁹⁻¹². Due to the high stability of the steroid skeleton, products derived from steroids can be detected in consolidated sediments far into an advanced stage of maturation. We have examined 11 recent sediments and suggest here that the Δ^2 -sterenes which they contain are degradation intermediates of the precursor sterols.

The characterisation of degradation intermediates, for example, unsaturated hydrocarbons, can bring a better understanding of the nature of the evolution pathways undergone by the sterols in the sedimentary environment, as well as useful information on the stages of evolution at which various degradation reactions take place¹³. Mono- and di-unsaturated steroid alkenes have been tentatively detected in various sediments, but these compounds have not been conclusively identified¹⁴⁻¹⁶.

We have now studied the unsaturated hydrocarbon fraction of 11 recent sediments—five from the sea of Norway, one from the Baltic sea, two from the vicinity of the Amazon estuary, one from the Cariaco Trench near Venezuela and two continental muds from the immediate vicinity of Strasbourg. The samples were frozen and freeze-dried immediately after collection. The dry samples were then extracted with freshly-distilled chloroform in a Soxhlet apparatus or under ultrasonics. When necessary the total extracts were desulphurised on active copper and separated by thin-layer chromatography (TLC) on SiO_2 with hexane elution. The unsaturated hydrocarbons (10–20% of the total saturated + unsaturated hydrocarbons) were further separated by Ag^+/SiO_2 TLC with hexane elution yielding 1–2 p.p.m. by weight based on

Fig. 1 Differential thermal analysis curves of calcite and dolomite obtained from artificial mixtures of *a*, 0.5% calcite; *b*, 6% calcite; *c*, 6% calcite; *d*, 6% dolomite; *e*, 0.5% dolomite; *f*, 6% dolomite by weight with calcined alumina. Determinations being made in furnace atmosphere conditions of static air (dotted line) or dynamic carbon dioxide (solid line). The much improved peak definition, resolution and detection limits are clearly shown by the curves determined in dynamic carbon dioxide.



dry sediment (standard precautions against contamination necessary in organic geochemical studies were taken throughout). Gas chromatographic (GC) analyses were carried out on 1% Dexsil-packed glass columns (2.5 m $\times$ 3 mm) and OV-101 and Apiezon L capillary columns (25 m $\times$ 0.5 mm i.d.) using a GC-mass spectrometry-computer set-up (LKB 9000 S/PDP 11E 10). In control experiments a solution of 70 mg of pure cholesterol in chloroform was treated with 50 g of a chloroform pre-extracted sediment under ultrasonics: no trace of Δ^2 -sterenes could be detected after extraction and separation following the analytical procedure described above.

We have identified, from the unsaturated fractions of the recent sediments studied, three major constituents (Fig. 1) as the C_{27} , C_{28} and C_{29} 5α H) Δ^2 -sterenes *a-c* by the following criteria: mass spectral fragmentation patterns identical with those of the corresponding Δ^2 -sterenes which we and others¹⁷ had prepared, and co-elution with the reference compounds (C_{27} and C_{29}) on two capillary columns (OV-101 and Apiezon L). The mass spectra of the Δ^2 -sterenes show significant differences from those of Δ^4 - or Δ^5 -sterenes, since the former are characterised by an important fragment at M-54 (loss of butadiene due to a retro-Diels reaction)¹⁸. Stereochemistry at C_{24} remains undetermined.

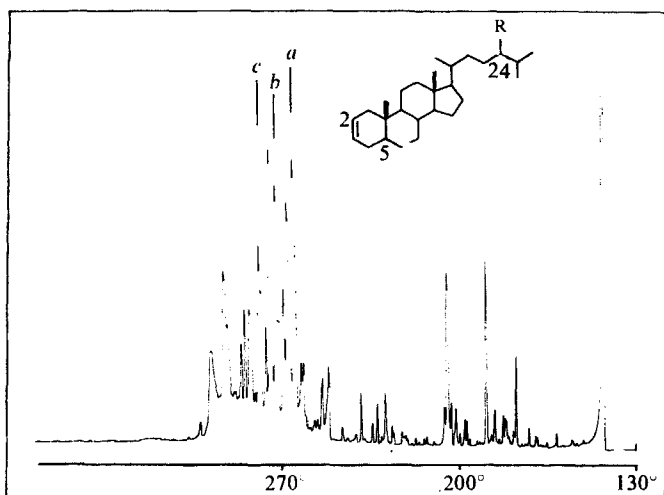


Fig. 1 Gas chromatogram of the unsaturated hydrocarbons of a recent marine sediment (5,000 yr) from the Cariaco Trench, near Venezuela. Conditions: OV 101, 25 m $\times$ 0.5 mm i.d., 130–270 $^{\circ}$ C, 3 $^{\circ}$ C min⁻¹. Peaks correspond to the Δ^2 -sterenes: *a*, R = H; *b*, R = CH₃; *c*, R = C₂H₅.

Recent results are good evidence for the microbiological reduction of Δ^5 -sterols into the corresponding 5α H (and 5β H) stanols in the first stages of diagenesis in surface sediments^{7,19,20}. The Δ^2 -sterenes (which have so far only been reported in non-fat dry milk¹⁷) may be formed in a further stage by dehydration of the corresponding stanols. In one of our samples, from the sea of Norway, which showed a slightly acidic pH, we noticed the concomitant presence of a series of $\Delta^{13(17)}$ -sterenes (C_{27} – C_{29}) with a backbone-rearranged skeleton. It is reasonable to assume that these compounds, which we have previously identified in several older shales, are formed from stanols by an acid-catalysed skeletal rearrangement through Δ^2 -sterene intermediates. This process has been clearly demonstrated by simulation experiments with cholesterol or Δ^2 -cholestene and montmorillonite clay²¹. Further studies, however, are still needed to confirm that the isolation of rearranged sterenes is really due to the observed low pH.

The identification of Δ^2 -sterenes in various recent sediments deposited in marine or continental environments suggests that these compounds are degradation intermediates of the precursor sterols, via the corresponding stanols, in the sedimentary environment. It is also possible that they arise directly from stanols, since

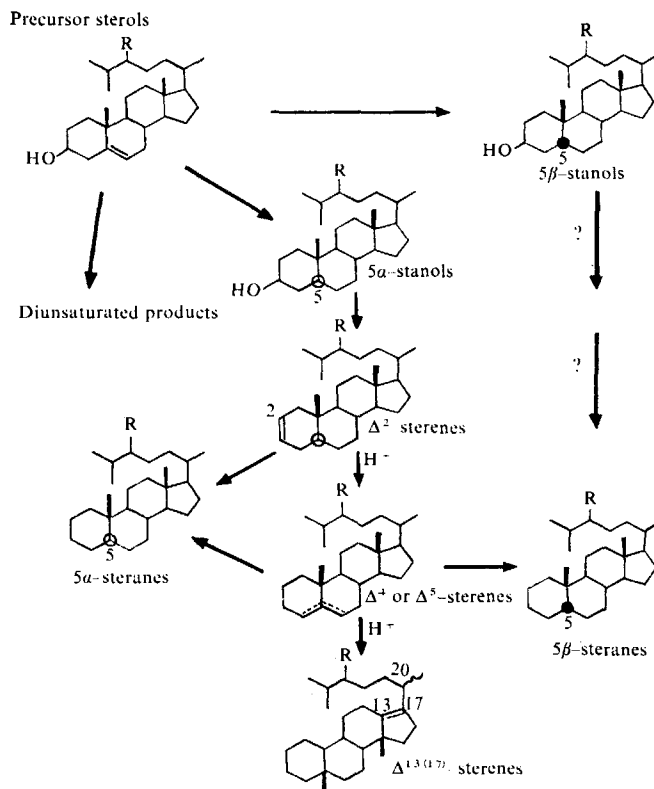


Fig. 2 Early transformations of sterols in the geological environment, based on laboratory and field results. Processes marked ? are only inferred (see refs 8, 9, 20, 21 and refs therein) R = H, CH₃, C₂H₅.

the latter have been detected in several living organisms⁸. These alterations start at a very early stage of diagenesis in surface sediments. These results, combined with our, and other authors' previous results, increase our knowledge of the first geochemical evolution pathways undergone by the sterols in the geological environment, as shown in Fig. 2.

This work constitutes a contribution to the 'Projet ORGON', an interdisciplinary approach study of recent sediments undertaken by several French laboratories. We thank ELF-Aquitaine for financial support.

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Received 29 March; accepted 8 August 1977.

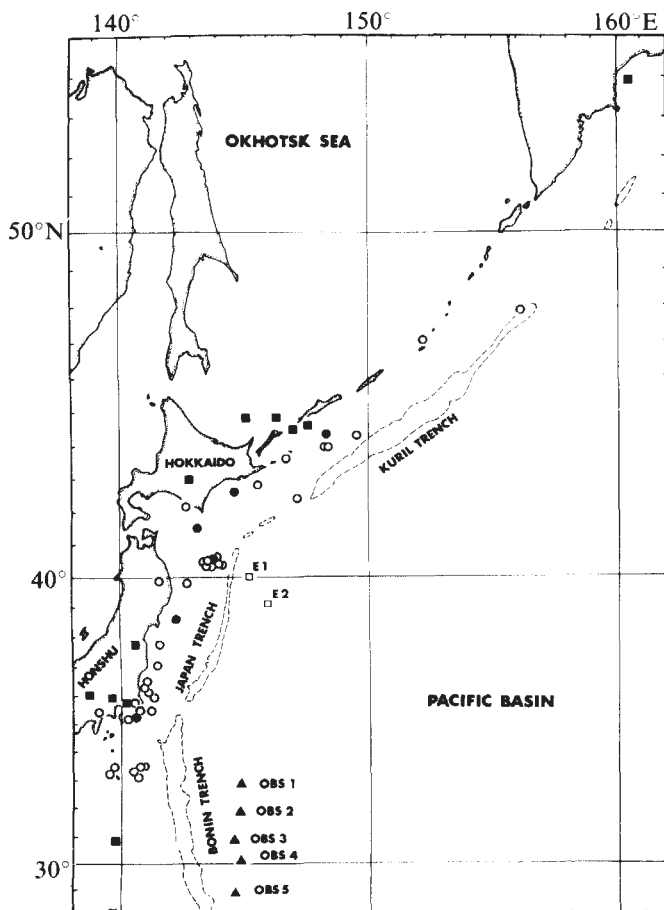
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High shear velocity layer in the upper mantle of the Western Pacific

SHEAR waves have been only rarely observed in explosion seismology studies at sea, so their precise velocities have not been extensively studied. An inversion method from seismic surface wave studies has been used to measure the shear wave velocities of the oceanic upper mantle¹⁻³. But, the values obtained, generally 4.6 km s^{-1} , are inherently an average of a path from a seismic source to a seismic recording site on land, so that the method can not give a 'pure' value for the upper oceanic mantle. Shear wave velocity is, however, important for the physical property of the upper mantle as well as for discriminating the minerals which make up the oceanic upper mantle. We describe here our measurements of apparent shear wave velocities, using 61 natural earthquakes with epicentral distances ranging from 400 to 2,900 km. Results indicate that the lower part of the oceanic lithosphere contains a large amount of garnet.

A linear array of high sensitive ocean bottom seismographs (OBSs) was positioned on the Western Pacific Basin, where the Pacific lithosphere is thought to be very old and about to subside (Fig. 1). The water depth is between 5,500 and 5,600 m. The linear dimension of the array is about

Fig. 1 A map showing the array of ocean bottom seismographs (▲) and precisely located epicentres of earthquakes, having focal depths shallower than 40 km (○), 41–70 km (●), and 71–190 km (■). Note that most of the wave paths from the foci to the OBS array pass through the upper mantle of the Pacific lithosphere (if we consider that the lithosphere is subducting north-westerly); hence the obtained apparent velocities represent those of Pacific upper mantle. □, 7- and 5-t explosions which were used for the explosion seismology study⁵ where P-wave structure has been obtained for the region.



400 km, so that the apparent velocities were accurately measured within the array⁴.

The observations were carried out during a two-week period in October 1974, when 61 shallow earthquakes were precisely located by various land seismograph networks (Japan Meteorological Agency, USGS, the seismological network of Sakhalin Complex Scientific Institute USSR, seismological networks operated by Tokyo University and Hokkaido University in Japan). Most of the earthquakes occurred along the Kuril trench and Japan trench which are, seismically, the most active regions in the world. Since the OBSs are sensitive, earthquakes with magnitude 3 or 4 were clearly recorded at distances of 1,000 or 1,600 km, respectively.

Our observed S travel times, from earthquake foci to the OBSs, are shown in Fig. 2. These are markedly smaller than those of the Jeffreys–Bullen travel time tables. The differences are 10–30 s for shallow earthquakes with 400 to 1,800 km range of epicentral distances. The results indicate that the structure of the oceanic upper mantle at the Western Pacific is unusual. Travel times, however, have inherent limitations in resolution since they are strongly affected by errors in their origin times which are given by seismograph networks on land.

Apparent velocities, which are measured from passing times of seismic waves which cross the OBS array and indicate the velocities of the rocks at the deepest points of seismic rays, are accurate, since they are free from errors in the origin times. Although they give useful information, the apparent velocities are the least sensitive quantity to the complexity of the structure.

The region west of the trenches probably has a complicated structure that includes a continental shelf overlying a subducting lithosphere. The structure may cause errors in the determination of hypocentres and deflections of seismic waves which are incident to the OBS array.

Some calculations on ray paths, using plausible velocity models for the region, reveal that the complexity of the region's structure cannot affect the obtained apparent velocities by more than 1%. The size of the error is the result of choosing earthquakes with small errors in focal positions for our analysis and also that the earthquake foci are very close, in a three-dimensional sense, to the plausible subducting lithosphere, which is considered to subduct towards the north-westerly from the trenches.

The apparent velocities were calculated from pairs of readings by adjacent OBSs, which are indicated by line segments in Fig. 2. Figure 3 demonstrates that V_s values are faster than 4.6 km s^{-1} . The average value, $4.88 \pm 0.12 \text{ km s}^{-1}$, is obtained from 59 data for shallow earthquakes, since calculated velocities seem to have little dependency on epicentral distances within the range of the figure. The small standard deviation suggests the accuracy of overall measurements of this analysis, which are composites of errors in source locations, errors in OBS locations, timing errors in OBS and the possible inhomogeneities of structures beneath the OBSs sites. The effect of incident angle to the array has been compensated.

The obtained value of S-wave velocities, which show a predominance at 4.9 km s^{-1} , in all probability corresponds to the P-wave velocity of 8.6 km s^{-1} , which has been obtained by a long-range explosion seismology study⁵ and apparent velocity measurements for P waves⁴, at the same region. Hence from a probable analogy to the P-wave structure which was measured by the explosion experiment, the measured S value, 4.9 km s^{-1} for the range 500 through 1,800 km of epicentral distances, indicates the S-wave velocities in layers from 60 km to about 150 km depths, except for the low velocity layer.

The existence of the low velocity layer, which is observed by the explosion experiment and apparent velocity measure-

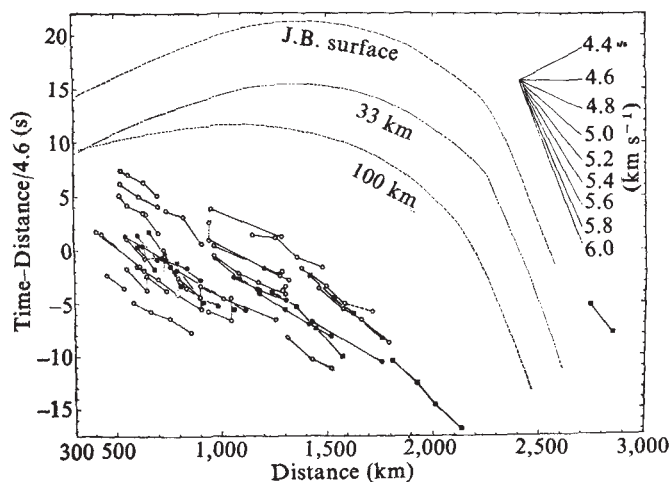


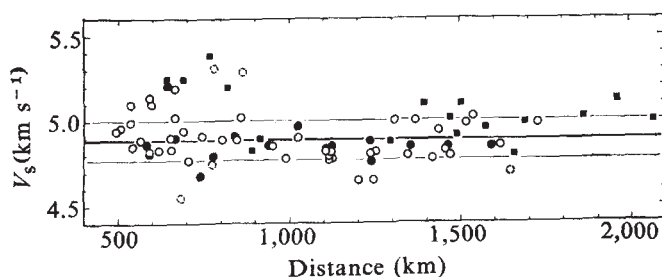
Fig. 2 Travel times of earthquakes, whose epicentres are shown in Fig. 1, observed by the OBS array. $\circ$, $\bullet$ and $\blacksquare$ as for Fig. 1. Each group of short line segments denotes an event of an earthquake. Travel times of Jeffreys-Bullen, for focal depths of 0, 33, and 100 km, are also shown. The observed travel times are remarkably faster. Vertical dotted lines are possible offsets.

ments for P waves, is also suggested by the present S-wave study. The effect of the low velocity layer beneath the oceanic lithosphere is demonstrated by offsets of travel times⁴. This is the case for S waves. The observed offsets are indicated in Fig. 2 by dotted lines. Although we could not obtain the V_s value in the low velocity layer since it is not observed as an apparent velocity, the thickness of the low S-velocity layer is estimated to be as thin as that of the low P-velocity layer which was measured to be less than 50 km (ref. 5), if we consider the travel time differences at the observed offsets. We conclude that the oceanic upper mantle at the Western Pacific mainly consists of 4.9 km s^{-1} layer, except for the uppermost 4.6 km s^{-1} layer with a probable small thickness and the low velocity channel which also has a small thickness. The depth of the bottom of the 4.9 km s^{-1} layer was not known accurately.

The V_s value of 4.9 km s^{-1} sets an important limitation on the candidate minerals which could make up the oceanic upper mantle. M. Kumazawa has measured V_p and V_s for the various minerals which have been considered as components of the oceanic upper mantles, under various temperatures and pressures (personal communication). A description of the pressure at the lower part of oceanic lithospheres has thus been obtained, although some ambiguities which are mainly effects of temperature and pressure, have been introduced. A three-component composite rock—the most probable composition for the oceanic lithosphere—is assumed (see Fig. 4).

Figure 4 indicates that pure olivine, pure pyroxene or a mixture of olivine and pyroxene fails to meet the seismo-

Fig. 3 Apparent velocities obtained from Fig. 2. Only shallow earthquakes with focal depths less than 40 km are used to obtain an average velocity of $4.88 \pm 0.12 \text{ km s}^{-1}$. $\circ$, $\bullet$ and $\blacksquare$ as for Fig. 1; broken circles are less reliable, data are probably contaminated by a possible inclusion of offsets in the span of the OBS array.



logically-obtained values, unless an impossible temperature, such as lower than 250°C , is assumed. Therefore, a considerable amount of garnet is necessary to explain the high velocities of P and S, despite the fact that garnet has not been widely accepted to be a major component of oceanic lithosphere (except by Ito⁶). The content of garnet in the lower part of lithosphere is also suggested by Fig. 4 to be probably more than half, possibly 60 to 80% of all the minerals present, under any assumption of temperature which has been generally accepted, that is $500\text{--}1,000^\circ\text{C}$.

Although the finer structure of the lower part of the oceanic lithosphere has not been elucidated in this study, a probable mechanism to compensate for the increase of V_p and V_s due to higher pressure towards the bottom of the

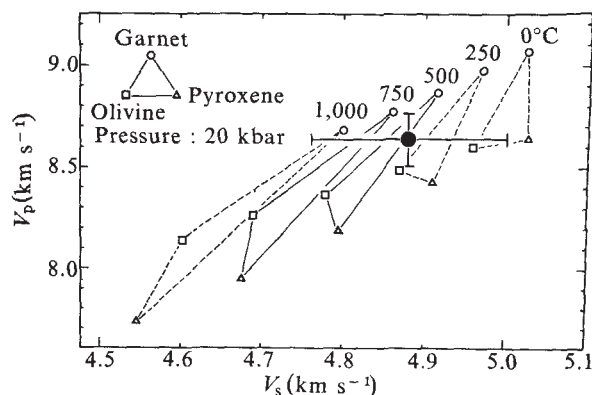


Fig. 4 Seismic wave velocities of the three-component composite rock at a pressure of the top of the high velocity layer in the oceanic lithosphere, for various conditions of temperature. The postulated components are garnet [70% pyrope ($\text{Mg}_3\text{Al}_2\text{Si}_3\text{O}_{12}$) + 30% almandine ($\text{Fe}_3\text{Al}_2\text{Si}_3\text{O}_{12}$)], pyroxene [90% enstatite (MgSiO_3) + 10% ferrosilite (FeSiO_3)] and olivine [90% forsterite (Mg_2SiO_4) + 10% fayalite (FeSiO_4)]. The measured velocities, $8.64 \pm 0.13 \text{ km s}^{-1}$ for V_p (ref. 4) and $4.88 \pm 0.12 \text{ km s}^{-1}$ for V_s , from ocean bottom seismometry are also shown.

oceanic lithosphere by the decrease of V_p and V_s due to increase of temperature has been suggested, which results in the fairly uniform values of V_p and V_s for a wide range of depths, that corresponds to a wide range of the epicentral distances (Fig. 3).

Another layer of 4.9 km s^{-1} which lies beneath the low velocity layer, may also be accounted for by the identical garnet-rich rock because of a similar compensation. But uncertainties of high temperatures and pressures effects in the range obstruct any definite interpretation.

We are planning another experiment in which OBS array will be placed perpendicular to that of the present study, which means an array perpendicular to the local magnetic anomaly: this should show whether or not V_p and V_s values larger than $8.6\text{--}4.9 \text{ km s}^{-1}$ can be obtained.

We thank Dr K. Ito for helpful discussions.

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Uranium series ages and late Quaternary uplift in the New Hebrides

CURRENT interest in geodynamics has stimulated research into late Quaternary uplift rates of the Earth's crust from uranium series age determinations of elevated coral reefs^{1,2}. Of particular interest is the subduction rate of the Australian Plate beneath the New Hebridean Island Arc along the New Hebrides Trench (Fig. 1) derived using the uplift rate of a pre-subduction lithosphere flexure near the south-eastern Loyalty Islands^{3,4}. We report here seven uranium series ages of elevated coral reefs in the New Hebridean Islands in the hope that estimates of uplift rate in the island arc will provide additional constraints for Quaternary plate-tectonic models.

The history of the New Hebridean islands since their formation in the Oligocene is relatively well known⁵⁻⁷. Before the Pliocene, the rocks deposited were largely basalt and volcanoclastic sediment. In the late Pliocene substantial deposition of reef limestone became common⁶, especially in the eastern and western belts of the archipelago indicating slow sinking of most of the archipelago for 1 or 2 Myr. Since about the mid Pleistocene, however, many islands of the group rose progressively above sea level, as is indicated by flights of raised reefs along their coasts.

Raised reef sequences have been described on Malekula⁸, Pentecost⁹, Efate⁷, and Malo Island¹⁰ (Fig. 1). The general appearance and sedimentary analysis of the reef capped terraces on Malekula suggest that they were formed by transgressive-regressive cycles, probably resulting from eustatic sea-level oscillations, combined with continuous uplift of the coastal section concerned⁸. Similar models, supported by uranium series age, have been advanced for raised reefs on Barbados^{11,12}, the Ryuku Islands⁵, the Huon Peninsula of New Guinea^{13,14} and Timor and Atauro in the Banda Arc³.

The ages presented here are from samples collected (by G.N.) on Efate and Malo Islands during May 1974. The raised reefs form good topographical features but they are rarely well exposed and road cuts are the best collecting sites. Heights were estimated by two pocket altimeters and these readings are consistent with contours of the 1:100,000 topographical maps published by the Institut Geographique National, Paris. Radiometric analyses have been carried out (by H.H.V.) using methods previously described². The results are shown in Table 1.

Although only a limited number of the elevated reef terraces yielded coral samples suitable for age determinations, all terrace levels recognised in the field are indicated in Table 1, to aid in the interpretation and correlation of dated terraces. The ages were calculated from the measured ²³⁰Th/²³⁴U ratios, assuming initial isotopic activity ratios of ²³⁴U/²³⁸U = 1.15 and ²³⁰Th/²³⁴U = 0^{2,11-14}. The ²³²Th present in the corals was negligible, and no corrections have been made for possible secondary addition of common ²³⁰Th.

On Malo Island, 40 km north of Malekula, five raised reefs are gently tilted to the east¹⁰. Four of these raised reefs are present along an east-trending track near Ana settlement and two raised reefs have provided ages. Another sample, Ma 1, collected from a track between Abountari settlement and Pic Malo is probably the same age as the highest raised reef on the Ana sequence. Unlike Malo, North-West Efate is cut by several late

Quaternary faults which trend westerly and north-westerly¹⁵. A Holocene raised reef is mapped¹⁵ and above this at least three raised reefs are recognised on air photographs between Creek Ai and the air strip. One of the samples (Efate X) is from the much dissected highest raised reef and samples Efate 235 and Efate 180 from north-west of Port Havannah, lie adjacent to a late Quaternary fault on the 'upthrow' side. Because of poor exposures and late Quaternary diastrophism, then, correlation of raised reefs developed in the New Hebrides is not yet possible. Also using altimeters for height measurement can lead to significant errors. Age assignment to undated raised reefs on the basis of sea-level maxima recognised elsewhere is therefore not reliable, and has not been attempted here.

There is little doubt, however, that the terrace represented by Malo LT-1 and Efate 20 corresponds to reef complex I of Huon Peninsula, dated at 5,000 to 9,000 yr¹⁴. Similarly, the raised reefs represented by samples Efate 330 and Ma 1, both dated at 134,000 yr, and Efate X and Efate 235 both dated at about 120,000 yr may well correspond to sea-level stands VIIa (140,000 yr) and VIIb (120,000 yr), respectively, of Huon Peninsula^{13,14}. The ages of Efate 330 and Ma 1 are judged to be more reliable than those of Efate X and Efate 235, on the basis of mineralogical criteria used in the evaluation of dated

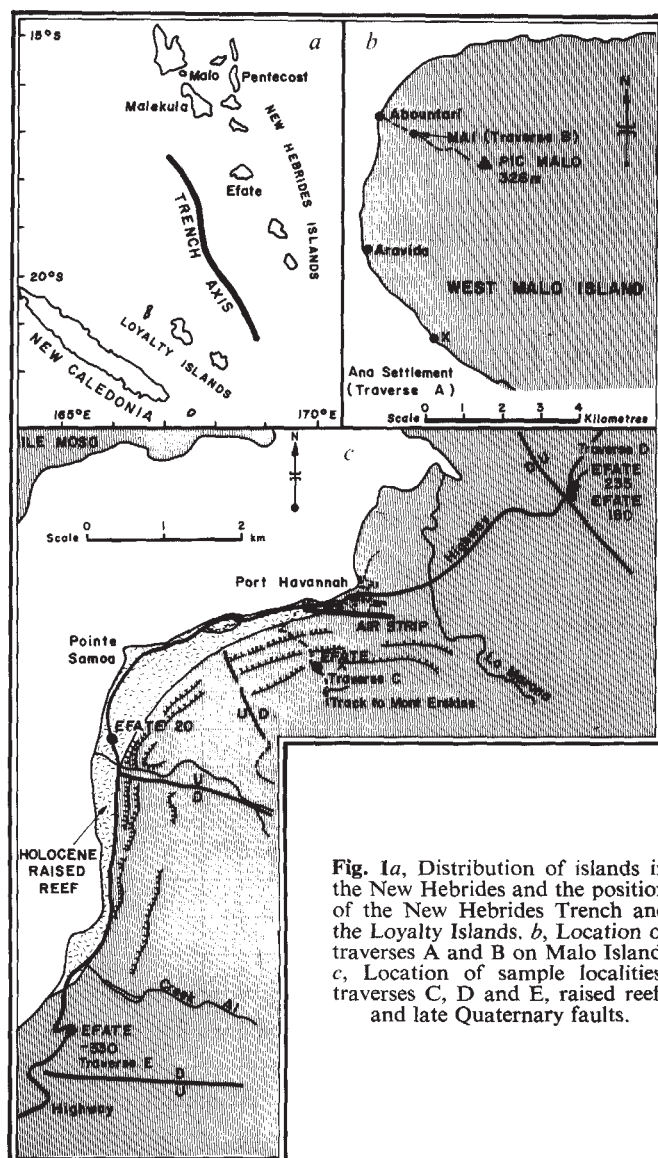


Fig. 1a, Distribution of islands in the New Hebrides and the position of the New Hebrides Trench and the Loyalty Islands. b, Location of traverses A and B on Malo Island. c, Location of sample localities, traverses C, D and E, raised reefs and late Quaternary faults.

Table 1 Raised reef elevations and radiometric ages of dated samples

Traverse	Elevation of terrace (m)	Sample no.	Calcite* (%)	U (p.p.m.)	U ²³⁴ /U ²³⁸	Th ²³⁰ /U ²³⁴	Age (× 10 ³ yr)
Malo A	94–98† 49–55 35–41 17 4.5	Malo LT-4B	< 2	2.57	1.08 ± 0.03	0.43 ± 0.02	60 ± 4
B	96	Malo LT-1 Ma 1	< 2 3	2.47 2.56	1.15 ± 0.03 1.10 ± 0.02	0.035 ± 0.004 0.72 ± 0.03	4 ± 0.5 134 ± 10
Efate C	c 100 c 75 c 40 6	Efate X	5	2.37	1.08 ± 0.02	0.67 ± 0.02	118 ± 7
D	c 140 c 100 72 55 c 40 6	Efate 235 Efate 180	< 2‡ < 2	2.80 2.20	1.09 ± 0.02 1.12 ± 0.01	0.68 ± 0.02 0.53 ± 0.02	120 ± 7 81 ± 4
E	c 230 c 170 c 130 100 c 75 c 45 6	Efate 330 Efate 20	< 2 < 2	2.94 2.56	1.13 ± 0.02 1.12 ± 0.02	0.72 ± 0.02 0.066 ± 0.005	134 ± 8 7 ± 0.6

The errors shown are based on counting statistics (+1σ).

*Based on X-ray diffraction analysis of representative coral sample after mechanical cleaning with dentist drill and/or ultrasonic vibration.

†Range in elevation between front and back of terrace.

‡Contained void filling micrite cement before clean-up.

samples^{2,14}. Efate X and Efate 235 may be suspect, the former because of its relatively high calcite content, suggesting incipient recrystallisation, the latter because it contained some secondary micrite cement which may not have been completely removed during the mechanical clean-up procedure. Furthermore, a distinction between the two age groups solely on the basis of radiometric ages may not be warranted, considering that the ages are not significantly different at the 95% confidence level. Two separate and stratigraphically well defined transgressions at 134,000 yr and 119,000 yr have also been recognised on the island of Atauro, north of Timor², supporting the view that the '125,000 yr sea-level stand' may in fact be the mean of two closely spaced, yet separate, stands of roughly the same elevation, the recognition of which depends on uplift rate and/or stratigraphic definition of a given raised reef sequence^{2,16,17}.

Because our data are limited, with only one age determination per individual terrace site, or none at all, we are not yet prepared to discuss the fine structure of palaeosea level in the New Hebrides over the past 140,000 yr. The ages given here for several of the raised reefs are useful, however, as they provide a more realistic time base for the late Pleistocene tectonic history of the area than has so far been available. For example, previous age estimates of the raised reefs on Malekula were obtained by assuming that the highest of the six tilted raised reefs present corresponds to the interglacial following the first cold period of the Pleistocene, hence it should be ~2 Myr old, with the ages of the other raised reefs, in descending order, calculated to be 1.8, 1.5, 1.0, 0.6 and 0.4 Myr on the basis of a steady uplift rate⁸. Mallick and Neef⁹ considered these age estimates far too old in comparison with the eustatic sea-level data from dated raised reefs on Huon Peninsula, a view which is supported by our much younger ages for terraces of similar elevation on nearby Malo Island, lying only 40 km from north-west Malekula.

The question of uniform or non-uniform uplift rate with time at a given location cannot be resolved without further field work and additional age determinations in the New Hebrides.

We propose that the mean uplift rate over the past 140,000 yr was at least 0.67 m per 1,000 yr on Malo, and between 0.55 and 0.77 m per 1,000 yr on North-West Efate—based on the data shown in Table 1 and the best estimates of palaeo sea levels at 120,000 and 134,000 yr (refs 2, 17). These values are not substantially different from those determined by similar methods in other active island arcs and orogenic belts along zones of lithosphere plate convergence^{1,2,11,13,14} but significantly higher than the uplift rate of 0.13 m per 1,000 yr estimated for the pre-subduction bulge west of the New Hebrides Trench¹.

We thank Dr D. I. J. Mallick for advice on potential sample locations and Mrs G. Halliday for drafting Fig. 1. Financial assistance to G.N. was provided by the University of New South Wales and the Mine Managers' Association of Broken Hill and to H.H.V. by the Australian Research Grants Commission.

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New evidence and possible origin of native iron in ophiolites of eastern Canada

THERMOMAGNETIC analysis has been used to detect traces of submicroscopic elemental iron in four bodies of Early Palaeozoic oceanic metabasalt (Fig. 1). The initial finding of iron in Newfoundland ophiolites¹ is believed to be the first terrestrial discovery of native ferromagnetic metal through magnetism. A few rock samples in these four areas have unusually high Curie points indicating pure or nearly pure iron. In one basaltic pillow, previous magnetic evidence² was confirmed by electron microprobe showing very fine Fe particles embedded in chlorite. We propose that such particles formed by reduction of olivine under water and became trapped in the chlorite host. Although native iron seems rare on Earth^{3,4}, further suboptical discoveries through rock magnetism may be predicted. Such findings could prove valuable in identifying ancient oceanic crust generated in a reducing environment.

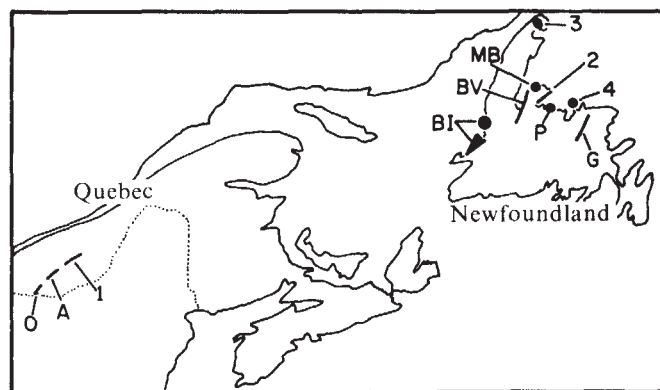


Fig. 1 Ophiolites and related rocks in Quebec and Newfoundland. A, Asbestos; BI, Bay of Islands; BV, Baie Verte; G, Gander River; MB, Ming's Bight; O, Orford; P, Pilleys Island. Bodies in which native iron was identified are numbered: 1, Thetford Mines; 2, Betts Cove; 3, Hare Bay; 4, Moreton's Harbour.

The Thetford Mines⁵ and Betts Cove⁶ ophiolites (Fig. 1, areas 1, 2) are closely similar⁷. The former is middle Cambrian or older and was emplaced in the early Ordovician⁵, while Betts Cove may have originated and been emplaced somewhat later^{8,9}. The early Ordovician^{10,11} transported ophiolite at Hare Bay (area 3) lacks gabbro and sheeted dikes. At Moreton's Harbour (area 4) an 8-km thick volcanic sequence is attributed¹² to Ordovician island arc volcanism. No iron has been detected outside of areas 1–4, nor in gabbros or ultramafics.

Curie points (T_c) were obtained from curves of high-field magnetic moment plotted against temperature (M – T). Powders were heated in vacuum² or air¹³ to 800 or 900 °C (Fig. 2a, c–e). Also initial susceptibility variation with temperature (K – T) of whole rock was measured in air¹⁴ (Fig. 2b). Iron contamination

can be ruled out as all specimens were prepared by rigorously avoiding contact with ferromagnetic materials.

Two of the heating curves (Fig. 2a, b) indicate magnetite, but all five show high T_c values in the range 755–775 °C. This corresponds to¹⁵ the Curie point of α -iron (770 °C) or of iron alloyed with less than 5% nickel (770–750 °C). The trends of the irreversible curves obtained in air seem to be better explained by oxidation of iron, initially to magnetite, than by α , γ phase transitions¹⁵. Figure 2c, d resembles curves for lunar samples which likewise may have been oxidised, though heated in a vacuum^{16,17}. The smaller saturation magnetisation of magnetite (4.80×10^5 A m⁻¹) than of iron (17.0×10^5 A m⁻¹) is consistent with this. Still, some samples became more magnetic upon cooling (Fig. 2b, e). Lunar samples (see ref. 17) show similar variations which suggest that grain-size effects are important. The large susceptibility increase on cooling in Fig. 2b may be due to superparamagnetic magnetite produced by heating the iron¹⁷.

Table 1 shows values of natural remanence, susceptibility and Koenigsberger ratio of Thetford Mines pillows. For any pillow zone the mean values are respectively three, one and two orders of magnitude less than world-wide averages for young drilled deep-sea basalts¹⁸. Low, widely variable Koenigsberger ratios (0.5 to 0.001) predominated also in Newfoundland. Very unevenly distributed secondary magnetite and/or iron could account for these findings.

Estimated elemental iron in samples does not exceed a few tenths of one weight percent. At Thetford Mines, iron was detected in 13 out of 51 thermomagnetic analyses made on 14 pillows (Table 1), with mean Curie point 756 ± 4 °C (standard deviation). In Newfoundland, 28 samples each had yielded at least one M – T curve showing significant iron (total 56 analyses). Mean Curie points are: Betts Cove pillows, 760 ± 11 °C (15 samples); Betts Cove dikes, 759 ± 7 °C (four samples); Hare Bay pillows, 761 ± 11 °C (six samples); Moreton's Harbour pillows, 750 ± 5 °C (three samples). Three reliable K – T curves, for Hare Bay (Fig. 2b) and Betts Cove, gave $T_c = 760$ – 775 °C.

At Laval University a Thetford Mines pillow that had produced M – T curves similar to Fig. 2a was shown by electron microprobe to contain small ($< 5 \mu\text{m}$), lamellar particles of 96–98% pure Fe; also chalcopyrite, sphene, magnetite and chromite. The opaques occur as minute inclusions in the fractures of former olivine microphenocrysts entirely pseudomorphosed by magnesian-chlorite. A single iron particle was just large enough to be seen with the reflecting microscope. In four samples from areas 2–4 in Fig. 1 having 'iron' but not 'magnetite' Curie points, microprobe analysis showed abundant sulphides, also sometimes limonite, hematite, iron oxyhydroxides or sphene, but neither magnetite nor Fe. This is compatible with the presence of extremely fine-grained iron.

Terrestrial nickel and iron has been described from less than 24 localities^{3,4} where nickel–iron containing 65–75% Ni always occurs only in serpentinised ultramafics. Nickel-poor iron ($> 90\%$ Fe), however, usually occurs in mafic rock^{3,4,19–22}. A second significant fact is that, wherever we inferred iron, the pillow lava or dike rock has undergone greenschist metamorphism. The pillow lava is of two types²³, a dark splititised metatholeiite rich in chlorite and a light green olivine metatholeiite rich in actinolite and chlorite pseudomorphs. At Thetford Mines, but not Betts Cove, native iron seems to be confined to the olivine metatholeiites².

Table 1 Magnetic properties and ferromagnetic constituents according to pillow zones (Thetford Mines, Quebec)

Pillow zone	<i>n</i>	$J_n (\times 10^{-3} \text{ A m}^{-1})$		$K (\times 4\pi \times 10^{-6})$		Range Q_n	Mean	n_{M}	n_{Fe}
		Range	Mean	Range	Mean				
Margin	15	1.15–4.25	1.95	18.0–57.3	45.2	0.03–0.13	0.05	15	0
Intermediate	22	0.56–3.26	1.42	19.9–35.9	31.1	0.05–0.14	0.08	22	9
Core	14	0.78–4.41	1.81	12.5–33.3	25.0	0.07–0.27	0.12	14	4

n, No. of whole-rock specimens (total 51) cut from 14 samples of pillow lava; J_n , intensity of NRM; K , initial susceptibility; $Q_n = J_n/KH$, natural Koenigsberger ratio, where H is the Earth's present field. Geometric means are quoted. One powder per whole-rock specimen was prepared for thermomagnetic analysis to identify the magnetic constituents (Fig. 2a, ref. 2); n_{M} , n_{Fe} , number of analyses indicating magnetite or (additionally) iron. Note that magnetite is inferred in all powders.

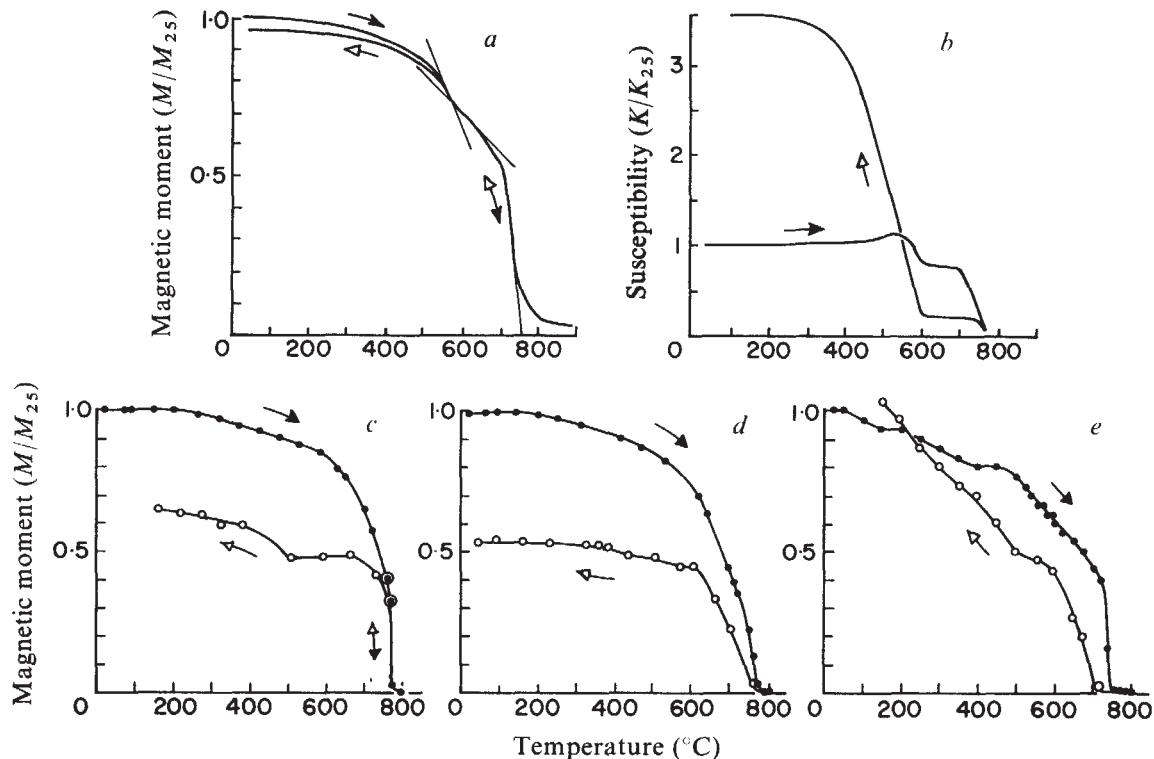
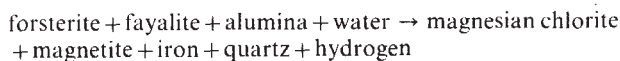


Fig. 2 Thermomagnetic curves for pillow lava from areas 1-4 (Fig. 1), normalised to 25 °C before heating. Specimens were heated and cooled in a vacuum of 10^{-3} torr (*a*) or in air (*b-e*). *b*, *K-T* curves (see text) for whole rock fragments up to 1 cm diameter, measured in a peak field $H = 0.031$ mT. *a*, *c-e* are *M-T* curves for coarse powders up to 1 mm, measured in fields of 0.10-0.13 T. Curie points (T_c) were obtained to better than ± 15 °C and are quoted to 5 °C. *a*, Thetford Mines, intermediate zone of pillow A (Table 1, ref. 2), showing two-component *M-T* curves. $T_c = 580$ °C, 755 °C. *b*, Hare Bay, intermediate zone of pillow HB 1, showing two-component *K-T* curves: $T_c = 595$ °C, 770 °C. *c*, Hare Bay, intermediate zone of pillow HB 1, *M-T* curves. $T_c = 770$ °C. *d*, Betts Cove, core of pillow BC 9, *M-T* curves. $T_c = 775$ °C. *e*, Moreton's Harbour, small pillow MH 33, *M-T* curves: $T_c = 755$ °C.

The iron could have originated (1) as dusts with magnetite in the rock ground-mass; (2) as reaction product between magnetite and iron sulphide in the ground-mass; or (3) as by-product of alteration of the olivine microphenocrysts. Mode (1) was not confirmed by microprobe and in any case the tiny, unprotected iron particles would have been rapidly oxidised. Mode (2) also seems unsupported since the iron identified by microprobe occurs without sulphide-magnetite; in the Newfoundland samples Fe tends to occur without magnetite; the association sulphide-magnetite is frequent in basalts while native iron is rare.

Alternative (3) assumes a paragenetic relationship between olivine and native iron. During serpentinisation of olivine, iron-related redox mechanisms may have controlled the formation in serpentine of magnetite and nickel-iron in equilibrium⁴. Serpentinisation itself might generate a highly reducing environment by the dissociation of water to provide the oxygen required to form magnetite, thereby releasing hydrogen^{24,25}. The same process can tentatively be applied to chloritisation of olivine in our rocks. The equilibrium assemblage resulting from the reaction



would comprise the minerals that actually replaced the olivine phenocrysts in the pillow lava. With sufficient hydrogen the magnetite can be further reduced to iron and water, which may explain its paucity in Newfoundland samples. The iron encapsulated in chlorite crystals would be protected against oxidation as long as the chlorite remained unaltered.

If this explanation is correct, then elemental iron should occur also in rocks of similar genesis elsewhere; for example, Curie points suggesting Fe have been reported²² from the greenschist facies of Archaean metavolcanics. Because of typically minute grain sizes, rock magnetism remains the most promising method for discovering native ferromagnetic metal.

We thank Dr M. B. Berubé, Dr L. G. Kristjansson, R. R. Pätzold, J. P. Ricbourg, Dr H. Upadhyay and T. Vallis for assistance. This work was supported by grants to E. R. D., R. L., and M. K.-S. from the NRC of Canada.

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Mesozoic sea floor off Dronning Maud Land, Antarctica

THE accreting margin between the African and Antarctic plates is now well understood between the Bouvet triple junction and Marion Island (Fig. 1). Published data obtained from the ridge axis near 1°W^1 , 15°E^2 , and 36°E^3 can be combined to yield a relative rotation pole at 11°N , 41°W with an equatorial half spreading rate of 0.8 cm yr^{-1} . Away from the spreading axis, very little is known apart from a narrow region to the north in the Mozambique Basin where Bergh and Norton³ have shown that the present spreading regime commenced at least as far back as the Late Cretaceous. This is the only region where transform fault traces have been extended for more than 100 km from the spreading centre and where magnetic lineations have been related to the present tectonic pattern. I describe here a group of lineations recently mapped in the Antarctic Basin off Dronning Maud Land, interpreted as being due to sea floor generated at the South-west Indian Ocean Ridge in the Early Cretaceous.

Two curtailed excursions in this area were carried out during the summers of 1976 and 1977. Figure 2 shows magnetic anomalies plotted perpendicular to ship's tracks. Anomaly identification was made by projecting the profiles on to 30°E (Fig. 3) and comparing with simulated profiles based on the reversal timescale of Larson and Hilde⁴. The Mesozoic sequence M-1 to M-9 (113 to 121 Myr.) is considered to be well matched, with M-2, M-4 and the diminutive M-6 showing up clearly. A spreading rate change from 2.0 to 1.5 cm yr^{-1} at anomaly M-5 time is apparent from the matching to the two theoretical profiles in Fig. 3. Anomalies older than M-9 are included only tentatively in the present interpretation.

The area surveyed is characterised by a generally featureless bottom topography with the regional depth sloping from 4 km at about 68°S to 5 km at 64°S . Limited seismic reflection profiling has revealed a flat-lying acoustic basement with sediment thickness of about 1.6 s of double travel time. A striking basement outcrop of up to 2 km above the surrounding sea floor is indicated by lineament F in Fig. 2. Its strike of 30° , based on three crossings, is exactly perpendicular to the mean azimuth of the observed magnetic lineations. Sea-floor depth is distinctly different on either side of this presumed fracture zone and is consistent with the expected older sea floor being on

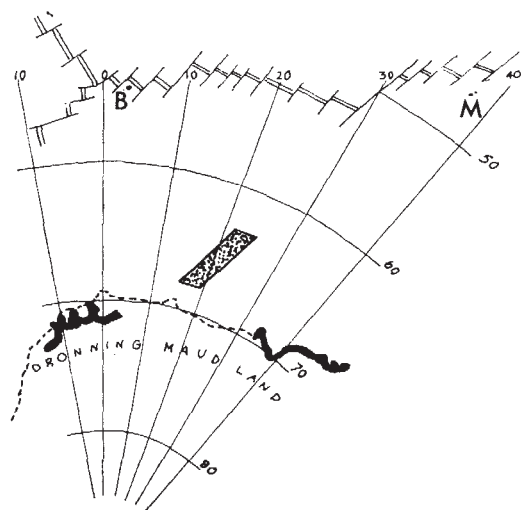


Fig. 1 Survey area (stippled) in Antarctic Basin. The South-west Indian Ocean Ridge and its junction with the Mid-Atlantic Ridge are shown schematically by double lines (sections of spreading ridge) and single lines (fracture zones and active transform faults). B, Bouvet Island; M, Marion Island.

the eastern side. A major west-facing fault scarp between 30° and 33°N in the Mozambique Basin, associated with Late Cretaceous spreading³ along the South-west Indian Ocean Ridge, is approximately co-polar (rotational) with fracture zone F, and has a similar depth contrast (500 m) across it. This is greatly in excess of calculations based on depth against age of ocean floor and could be related to a spreading style in which the two inward-facing walls of a deep transform fault crack move in opposite directions. The minor lineament f in Fig. 2 marks the eastward termination of identifiable magnetic anomalies, as indicated on profiles D and E in Fig. 3.

Magnetic anomaly and fracture zone trends reported here are consistent with their being initiated at the presently active ridge near Marion Island. More data will, of course, be required

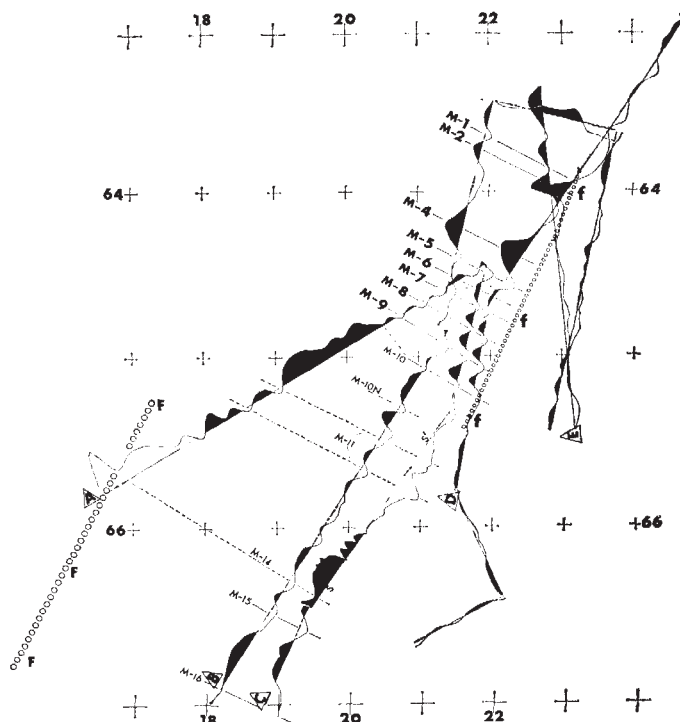
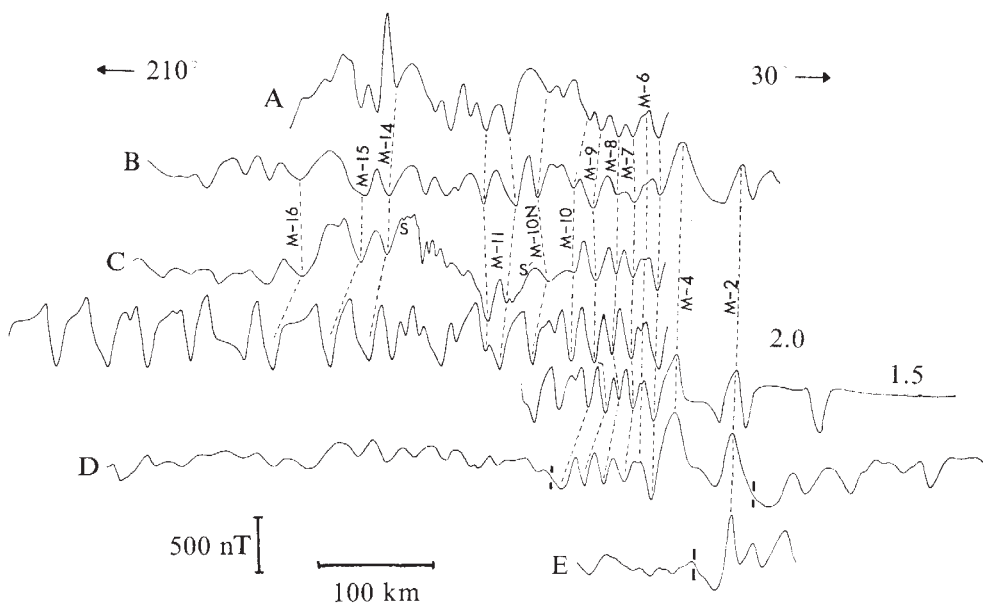


Fig. 2 Total field magnetic anomalies plotted perpendicular to ship's tracks after removal of International Geomagnetic Reference Field. Positive anomalies in black. Lineaments F and f were based respectively on a prominent basement ridge and the eastward termination of identifiable anomalies. Portion S-S' of profile C was surveyed during a magnetically disturbed period. Navigation by satellite and VLF Omega.

between the ridge and the M-sequence of anomalies before an unequivocal tie-in can be demonstrated. In Fig. 4 anomalies M-1 to M-9 as well as the lineaments F and f have been rotated by 20.5° about the present Africa-Antarctica rotation pole to bring anomaly M-1 close to the active accreting margin. Fault scarp F is nearly co-linear with the first fracture zone to the west of the Prince Edward fracture zone while lineament f coincides with an area of present dubious ridge signature which Bergh and Norton³ suggest might be an incipient transform fault. The ridge sections shown in Fig. 4 are based on data obtained subsequent to that included in their paper. The short section across 36°E is almost orthogonal to the Prince Edward fracture zone and is separated from the definitely oblique section ($37-38^\circ\text{E}$) by a nearly 50-km wide region where no central magnetic anomaly is apparent. The close correspondence between present and Mesozoic spreading might be fortuitous—it might also point to a persistent localised upper mantle property.

Fig. 3 Magnetic anomalies projected on to assumed spreading direction (Profiles A to E) and theoretical profiles for half spreading rates of 1.5 and 2.0 cm yr⁻¹. Simulations and anomaly numbering were based on the Larson and Hilde⁴ reversal time-scale with two-dimensional blocks of magnetised (1000 nT) sea floor trending 120° between depths 4.5 and 5.0 m. Vertical bars on profiles D and E mark the termination of identified anomalies and form the basis for the lineament f in Fig. 2.



As a working hypothesis, it is reasonable to assume that rifting along this segment of the ridge has proceeded in a remarkably stable way since the Early Cretaceous. Spreading rates have varied considerably and sections of ridge have changed orientation³ but the average pole describing the relative plate motion is very close to the present pole. Extrapolation of the Mesozoic spreading rate and direction back to the 2-km isobath off Dronning Maud Land dates the onset of spreading as roughly 150 Myr. or Late Jurassic. This slightly pre-dates estimates^{5,6} of the initial rifting of eastern Gondwanaland and is in very good agreement with the proposed early separation rate⁵ for that region.

This work was supported by the South African National Committee for Oceanographic Research. The M.V. RSA was

made available by the Department of Transport. I thank Captain E. Funk and his officers and crew for their cooperation and members of South African Antarctic teams and the Pretoria University Mammal Research Institute who assisted with watch-keeping.

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Received 16 May; accepted 13 July 1977.

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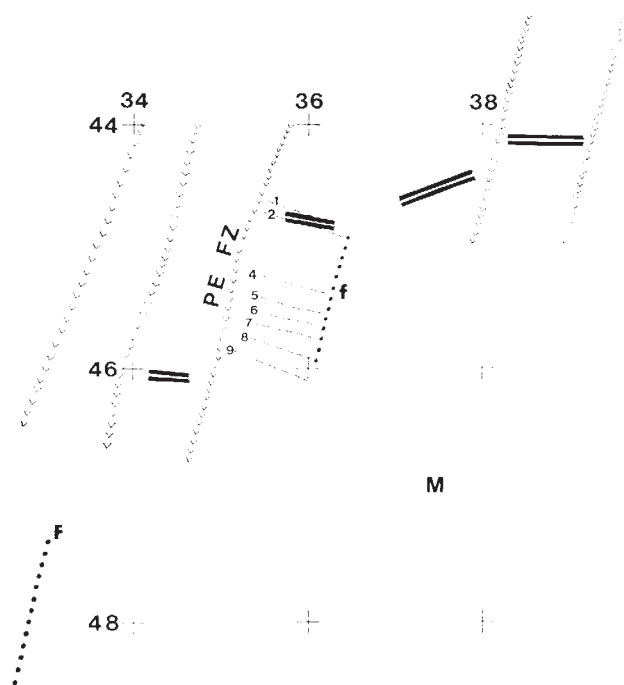


Fig. 4 Anomalies M-1 to M-9 and lineaments F, f rotated anti-clockwise by 20.5° about the present Africa–Antarctica relative rotation pole at 11°N, 41°W. Four sections of presently accreting plate margin are shown as thick double lines and the lines of vees represent fracture zones mapped by Bergh and Norton³. M, Marion Island.

Unusual diatom off the coast of south-west England and its effect on fishing

AN unfamiliar diatom was observed in the local townettings taken by research vessels of the Plymouth Laboratory towards the end of January 1977. It was a large member of the genus *Coscinodiscus*, but a species not previously known in the English Channel. The mucilage produced by this diatom was so abundant as to restrict trawling in some areas off Plymouth.

The valve pattern of the diatom resembled that of some forms of *Coscinodiscus concinnus* Wm Smith¹ with the valve having a clear centre, but the diameter was greater than that recorded for *C. concinnus* in the English Channel. It was unlike any other species of *Coscinodiscus* recorded for the western English Channel in that the 50-μm deep valve mantle met the valve face at right angles. As the valve itself was almost flat the diatom had a markedly rectangular outline in girdle view. A search of the literature showed that this diatom was probably *Coscinodiscus nobilis* Grunow. Our material agrees essentially with the description and illustration of *C. nobilis* given by Simonsen² and it agrees with the material of Grunow designated as lectotype by Simonsen². Grunow³ described this species from the Java Sea and it has only otherwise been recorded from the

Indian and Pacific Oceans⁵, although there are two doubtful records from the east coast of England⁶ and one from Heligoland⁷.

As the diatom was apparently increasing in numbers near Plymouth sampling was initiated in which 10 l samples were poured through a townet of 170- μ m mesh and the trapped diatoms counted. The diatom was successfully cultured in the laboratory and it divided every 2 d at 18 °C and every 3 d at 13 °C. In culture this diatom produced much more mucilage, which collected on the bottom of the culture vessel, than other species of *Coscinodiscus* we have cultured in similar conditions. Workers on our research ships complained that townets were becoming clogged with mucilage during sample collection.

During a hydrographic and biological cruise in the western English Channel on 19–21 April, the distribution of the diatom was plotted from townet samples (Fig. 1). On 1 May 1977 Dr A. J. Southward took a sample from 48°13'N, 08°11'W which was rich in this diatom, showing that it was abundant further west than we had sampled.

By the end of April the Fishery Officer at Plymouth was receiving complaints from the Plymouth fishermen that their trawls were becoming clogged or broken by a very heavy grey slime. The slime made hauling difficult and prolonged washing or air drying did not completely remove it. The slime was most prevalent on the sea bed in areas where the unusual diatom was most abundant.

Light microscopy showed that the slime was heavily loaded with clay particles and the insoluble remains of plankton organisms, such as the skeletons of silicoflagellates. The slime and the mucilage from the diatom were shown to be of the same chemical structure.

The diatom had reached high numbers for a cell of this size and had produced copious mucilage which, in sinking, had accumulated the insoluble skeletons of plankton organisms and, on the sea bed, had collected mineral particles which increased its volume and density. From our cultures we estimated that for a given volume of cells, there was two to three times the volume of mucilage present. Counts from seawater samples

taken off Plymouth were about 100 cells per l for several weeks in April. As the water column was completely mixed at that time, we assume that the diatoms were distributed through the whole 50 m of the water column. With a mean cell volume of 0.01 mm³ per diatom, 150 ml of slime could accumulate on each square metre of seabed. The slime would be concentrated in certain areas of the seabed by tides and the act of trawling would also accumulate it until it caused inconvenience to the fishermen. If a trawl with a 16 m spread were towed at 3 knots for 1 h it could sweep up about 5 m³ of slime. Even if more than half the slime passed through the net the accumulation on a 3-h tow would be considerable. Furthermore, although slime would pass through the net initially, once it had started to adhere to the meshes the percentage retained would increase rapidly. The volumes given here are minimal as the calculations are based on the mucilage produced by the diatoms and the accumulation of large quantities of bottom material in the slime would considerably increase its volume and density.

There are two published records of exotic diatoms appearing in European waters and forming a major part of the phytoplankton. *Biddulphia sinensis* Grev. was described from the China Sea⁸ and its first arrival and subsequent spread was recorded⁹. This species is now a prominent contributor to the winter and spring phytoplankton of the western English Channel. The second species, *Pleurosigma planctonicum* Simonsen, was first described² from the Indian Ocean. Simonsen collected his material in 1964–65 and we found this species in the western English Channel in 1966 (ref. 10). *Pleurosigma planctonicum* became dominant in the Plymouth area in 1973 and is still present in the English Channel, although its occurrence is sporadic. In neither case were there any reported effects other than the displacement of native species by the new arrivals.

We cannot offer an explanation as to how *Coscinodiscus nobilis* reached the English Channel. By mid-May, numbers had declined, although the species had not completely disappeared, and, by the end of May, there were no reports of slime clogging nets. Whether this diatom together with the inconvenience to trawling caused by its mucilage will become a regular feature of the spring phytoplankton in the English Channel remains to be seen.

We thank Dr Elizabeth Percival of Royal Holloway College, London, for analysing the mucilage and slime, Mr C. Knott and the crew of R.L. Gammarus for taking samples, Dr A. J. Southward for samples from RRS Shackleton and Mr W. Williams, District Fishery Officer, Plymouth, for information on the occurrence of the slime on the fishing grounds.

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Received 15 June; accepted 23 August 1977.

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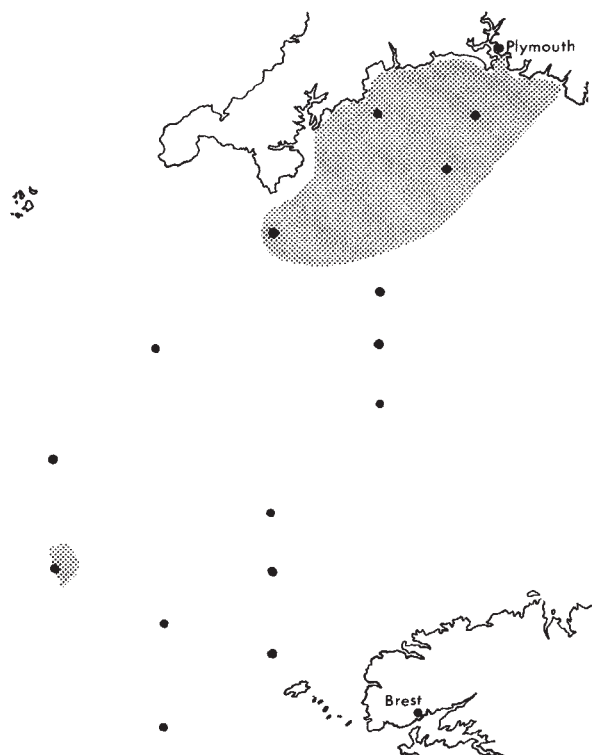


Fig. 1 Distribution of *Coscinodiscus nobilis* Grunow in the western English Channel, 19–21 April 1977. Shaded area, *C. nobilis* present in townet samples. ●, Stations worked.

Pattern regulation and transdetermination in *Drosophila* imaginal leg disk reagggregates

WHEN *Drosophila* imaginal disk tissue is cultured *in vivo*¹, the proliferating cells can adopt new fates within the limits of a single disk ('pattern regulation'^{2,3}) or may even produce structures normally derived from a different disk ('trans-determination'⁴). Much of the pattern regulation occurring in

imaginal disk fragments may be controlled by interactions between cells that come together during wound healing^{3,5}. I report here further studies on the role of cell interactions in pattern regulation and transdetermination, by means of dissociation and reaggregation of specific disk parts. The data confirm the importance of cell interactions for pattern regulation, and suggest that interactions between cells with large positional disparities may also be crucial for the stimulation of transdetermination.

We have previously studied pattern regulation and transdetermination in non-dissociated male foreleg disk quadrants (ref. 6 and unpublished results), and in identical fragments which were dissociated together with an excess of heavily irradiated wing disks ('feeding layer') and then cultured as 'mixed reaggregates'⁸.

In intact fragments, distal transformation⁹ (as judged by the regeneration of claws, which represent the distal tip of the adult appendage; Fig. 1a) occurred only in *UM*⁻ and, with a lower frequency, in *LM*⁻ quadrants (Table 2; for abbreviations see Fig. 1), whereas in mixed reaggregates, cells from all four quadrants frequently underwent distal transformation. Furthermore, although among the intact fragments only the *UM*⁽⁻⁾ quadrants transdetermined, cells from *UM*⁽⁻⁾ and *UL*⁽⁻⁾ quadrants underwent extensive transdetermination in mixed reaggregates. Since *LM*⁽⁻⁾ and *LL*⁽⁻⁾ cells failed to transdetermine in either situation, the hypothesis was advanced that only certain cells in the upper half of the male foreleg disk are capable of transdetermination¹⁰ (Fig. 1b). The more frequent distal transformation and transdetermination in mixed reaggregates could not be explained, however. It seemed possible that the initial destruction of the tissue organisation had caused both events, but I show here that they were stimulated mainly by interactions of the leg disk cells with 'feeding layer' cells present in the mixed reaggregates.

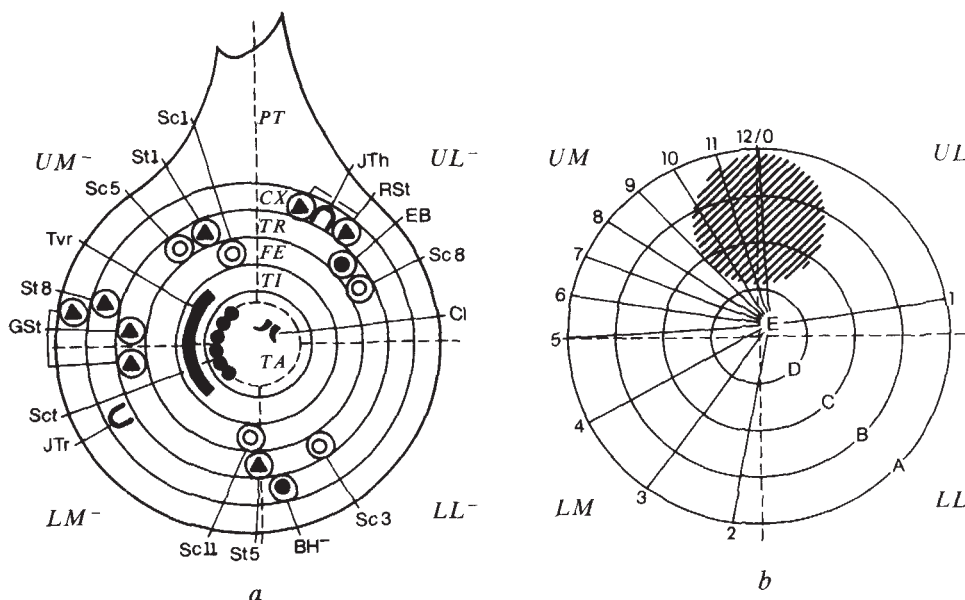
Drosophila male foreleg disk quadrants were dissociated, reaggregated, and cultured as 'pure reaggregates' (that is, without 'feeding layer'). In each experiment 120 quadrants (lacking most of the presumptive tarsal material; Fig. 1a) from third instar disks were dissociated with a microstirrer in a 0.05% trypsin solution⁷. The degree of dissociation varied as reported earlier⁸, clumps comprising up to about 20 cells occurring regularly. The reaggregated cell mass was divided and injected into fertilised adult females and cultured for 8 d at 25 °C. The implants were then transplanted into host larvae for

metamorphosis. Since most implants had to be cut into several pieces for this injection, the average number of larvae injected with material from one adult host represents a rough measure of the proliferative activity of the cells (Table 1). The death of injected larval hosts meant that a variable amount of material was lost in every experiment (Table 1). The differentiated implants consisted of a mass of vesicles, with each vesicle usually containing structures of a single leg segment. The number of vesicles typical for each leg segment was determined, and the cuticular landmarks (Fig. 1a) were scored. The implants were also examined for transdetermined structures. The data are presented in Table 1; Table 2 summarises the data available (refs 6, 8, 11, and this study) on pattern regulation and transdetermination in the male foreleg disk following different experimental treatments.

The main findings for pure reaggregates are (Table 1): (1) Distal transformation, as judged primarily by the regeneration of claws, but also by a comparison of the number of vesicles per leg segment (compared with control experiments, Table 1b), was very rare, even in *UM*⁻ reaggregates; (2) Regeneration of structures missing in a circumferential direction occurred rarely, if at all, in *LM*⁻, *LL*⁻ and *UL*⁻ reaggregates, and even in *UM*⁻ reaggregates only a few cases were observed; (3) Transdetermination was only observed in *UM*⁻ reaggregates, where two small wing-blade spots were found; (4) The rate of proliferation of the *LL*⁻ cells was very low, confirming earlier findings^{6,8}.

The rarity of both distal transformation and transdetermination in pure reaggregates contrasts sharply with the behaviour of identical leg disk cells in mixed reaggregates (Table 2). The data indicate that the high frequency of both events in mixed reaggregates did not result from the dissociation procedure *per se*, but rather from interactions between leg disk cells and 'feeding layer' cells. A specific effect of the irradiation of the 'feeding layer' cells on leg disk cells was ruled out by experiments in which *UM*⁻ quadrants were dissociated together with unirradiated wing disks. Both distal transformation and transdetermination were frequent in such reaggregates (Table 1b, D). The disk type of the 'feeding layer' tissue was also irrelevant to the behaviour of the leg disk cells. In mixed *UL*⁻ reaggregates containing either antenna disk or male foreleg disk 'feeding layers' (Table 1b, B, C) distal transformation and transdetermination occurred frequently, and the transdetermined structures were almost exclusively of wing disk type, as with

Fig. 1. a, Simplified fate map of the *Drosophila* male foreleg disk (after Schubiger¹⁸). Leg segments: PT, prothorax; CX, coxa; TR, trochanter; FE, femur; TI, tibia; TA, tarsus. Cuticular landmarks: ▲, groups of sensilla trichodea (St1, St8, GST, St5, RSt); ○, groups of sensilla campaniformia (Sc1, Sc5, Sc11, Sc3, Sc8); ●, single conspicuous bristles (BH⁻, EB), or sex comb teeth (Sct); JTr, JTh, joints; Tvr, transverse rows of bristles; Cl, claws. — — —, location of cuts. *UM*⁻, *LM*⁻, *LL*⁻, *UL*⁻, upper medial, lower medial, lower lateral and upper lateral quadrants, respectively, lacking their corresponding 'endknob' parts (which contain most of the tarsal material^{11,18}). *UM*⁽⁻⁾, *LM*⁽⁻⁾, *LL*⁽⁻⁾, *UL*⁽⁻⁾ in the text indicate that experiments with quadrants both containing and lacking their 'endknob' portions had been carried out. b, Polar coordinate model specifying positions in the *Drosophila* male foreleg disk (modified⁶ after French *et al.*³), and approximate location of the cells capable of transdetermination in the disk¹⁰ (hatching). 1–12, Circumferential positional values; A–E, radial positional values.



reaggregates containing wing disk 'feeding layer'⁸. It thus seems that cells from different imaginal disks exert similar effects on pattern regulation and transdetermination in intermixed leg disk cells.

In pure reaggregates, the cells of all quadrants chiefly formed structures characteristic for their region of origin (Table 1). This indicates that dissociated cells and cell clumps retain their positional information⁷. The mixed reaggregates, however, clearly show that these cells or cell clumps can, in appropriate conditions, be stimulated to acquire specific new developmental programmes. What factors caused identical cells to behave differently in different experimental conditions? The polar coordinate model of French *et al.*³ postulates that pattern regulation in *Drosophila* imaginal disks is controlled by interactions among cells carrying disparate positional information², brought into contact by wound healing or mechanical intermixture. Such disparities are thought to induce proliferation which, in accordance with specific rules, leads to the intercalation of missing positional values. According to this model the regeneration of a complete field circumference, as well as distal transformation, are possible only if a blastema possesses the majority of the circumferential positional values. Since in mixed reaggregates cells from all quadrants underwent distal transformation, the possibility was considered that the pattern regulation rules might not apply in reaggregates⁸. The rarity of distal transformation in pure reaggregates (Table 1), however, indicates that this is not the case, and suggests that the rules are still followed by dissociated and reaggregated cells and cell clumps. (It should be noted, however, that the polar coordinate model cannot explain the rare cases of distal transformation in pure *LM*⁻ reaggregates, nor the observed instances of distal transformation in intact *LM*⁻ quadrants and lateral halves; see Table 2 and ref. 11.) A more likely explanation for the behaviour

of the leg cells in mixed reaggregates is that they interacted with 'feeding layer' cells possessing non-homologous positional values⁸. In this way, complete circumferential sequences of positional information could be generated and allow distal transformation to occur. Evidence for communication between non-dissociated leg and wing disk tissues, resulting in the stimulation of intercalary regeneration⁵ in intermixed fragments (highly irradiated fragments being as effective in stimulation as unirradiated ones¹²) has now been obtained (ref. 13, and unpublished results of P. Adler, M. Fain and S.S.). These findings thus support the concept of a common mechanism of positional specification in different imaginal disks^{2,3,14}.

The high regenerative potentials of intact *UM*⁻ fragments led to this disk region being ascribed the majority of the circumferential positional values³ (Fig. 1b). Pure *UM*⁻ reaggregates, however, showed only rare regeneration (Table 1), a disparity which can be explained in terms of the polar coordinate model³. In intact *UM*⁻ fragments, wound healing always juxtaposes the two radial cut surfaces^{3,15}, thus creating the conditions necessary for the intercalation of the missing circumferential values ('shortest intercalation rule'), followed then by distal transformation ('complete circle rule'). In pure *UM*⁻ reaggregates, however, the cells have to become confronted by chance with cells showing a positional disparity corresponding to about half of the circumferential values, in order to regenerate complete circles which could then permit distal transformation. Clearly, the probability of such an event is quite small, especially since the *UM* region probably possesses only slightly more than half of the circumferential values⁶. In mixed reaggregates, however, cells from all quadrants would frequently confront 'feeding layer' cells that have sufficiently disparate positional values to allow the formation of complete circles followed by distal transformation.

Table 1 Structures formed by dissociated and reaggregated cells of the male foreleg disk quadrants, without (a) or with (b) intermixed 'feeding layer' cells, after an 8-d culture period in adults

Quadrant tested*	'Feeding layer'†	Size of grown impl.‡	Diff. impl. (recov. rate)§	Leg structures¶												Transdetermination
				<i>n</i>	PT lm	<i>n</i>	CX lm	<i>n</i>	TR lm	<i>n</i>	FE lm	<i>n</i>	TI lm	<i>n</i>	TA lm	
(a) <i>UM</i> ⁻	-	4.2	26 (49%)	21	GSt(5)	27	St8(11) JTr(5) BH-(3) JTh(3) RSt(8)	9	Sc5(7) Stl(3) GSt(6) St5(2) Sc3(2) Sc8(3) EB(1)	12	Sc1(3) Sc11(1)	21	Tvr(13)	11	Sc1(24) Cl(11)††	< 1%
<i>LM</i> ⁻	-	4.5	17 (63%)	10	GSt(6)	20	St8(9) JTr(4) BH-(6) BH-(2)	16	GSt(2) St5(10) Sc3(9)	16	Sc1(1) Sc11(8)	32	Tvr(114)	36	Sc1(440) Cl(5)††	
<i>LL</i> ⁻	-	1.0	3 (50%)			2					7		1			
<i>UL</i> ⁻	-	3.2	25 (78%)	12		24	JTh(3) RSt(6)	2	Sc8(2) EB(1)	44			53			
(b) A. <i>LM</i> ⁻	W**	5.3	14 (28%)	4		1		1	GSt(1)	7	Sc11(3)	11	Tvr(9)	41	Sc1(82) Cl(216)††	
B. <i>UL</i> ⁻	A**	4.0	10 (34%)	1		2				5		5		9	Cl(13)††	60%
C. <i>UL</i> ⁻	L**	4.2	24 (48%)			2	JTh(1) RSt(2)			2		6		24	Cl(51)††	85%
D. <i>UM</i> ⁻	W	16.4 ‡‡	56 (68%)							6		6		30	Sc1(95) Cl(41)††	10%

*Abbreviations as in Fig. 1a. Number of experiments performed: a, two experiments for each quadrant; b, A-D, one experiment each. Number of quadrants used per experiment: a, 120; bA and bD, 50; bB and bC, 10. Genotypes¹⁹ used: in a, bA and bD, equal numbers of *mwh* *e*¹¹ and *y v f mal*; *bw*; in bB and bC, *mwh* *e*¹¹.

†W, Wing disks; A, antenna disks; L, male foreleg disks. **, Disks irradiated with 17.5 Kr (ref. 8). Number of disks added: bA and bD, 20; bB and bC, 70. Genotypes¹⁹ used: bA and bD, *y*; bB and bC, *y f*^{3a}.

‡Value represents average number of larvae needed as hosts for the material recovered from one adult host.

§Recovery rate represents the percentage of injected implants recovered as differentiated implants.

¶Abbreviations as in Fig. 1a. *n*, Number of identified vesicles, printed in bold. In parentheses, number of times a certain landmark (lm) was encountered; underlining indicates that this landmark maps distinctly outside the quadrant tested.

||Percentage of total differentiated area occupied by transdetermined material (see ref. 8).

††Number of tarsal vesicles in which claws were present and (in parentheses) maximal number of claws observed in a single vesicle: I, *UM*⁻ 4(4); *LM*⁻ 2(3); bA, 34(16); bB, 9(2); bC, 24(4); bD, 22(5).

‡‡Only the material from 5 out of 16 adult hosts was subjected to metamorphosis.

Table 2 Pattern regulation and transdetermination by cells of the *Drosophila* male foreleg disk quadrants using different experimental conditions

Type of cell fate alteration	Experimental condition*	Quadrants†,‡				Ref.
		<i>UM</i> ⁻	<i>LM</i> ⁻	<i>LL</i> ⁻	<i>UL</i> ⁻	
Regeneration in circumferential direction	F	+++	—§¶	—§	—§	6, 11 8
	R, mixed	+	—	—	—	
	R, pure	+	—¶	—	—	
Regeneration of claws (distal transformation)	F	++	+	—	—	11 8
	R, mixed	+++	++++	+	+++	
	R, pure	+	+	—	—	
Transdetermination	F	+++	—	—	—	6, 11 8
	R, mixed	++++	—	—	++++	
	R, pure	+	—	—	—	

*F, Intact fragments; R, reagggregates.

†Abbreviations of quadrants as in Fig. 1a.

‡The symbols —, +, ++, +++, ++++ give a rough comparison of the frequencies or extents of pattern regulation or transdetermination, graded from — (not observed) to ++++ (observed in very large amounts). For the exact data see the literature cited.

§*LM*⁻, *LL*⁻, and *UL*⁻ quadrants, instead of regenerating missing structures, produce mirror-image duplications: *LM*⁻, +++; *LL*⁻, +; *UL*⁻, ++ (unpublished results, and see ref. 6).¶*LM* cells might be able to regenerate certain structures of the *LL* quadrant⁸.||Fraction of cultured quadrants (lacking their corresponding 'endknob' part¹¹) with claw regeneration: *UM*⁻, 14/30 (ref. 11); *LM*⁻, 3/19; *LL*⁻, 0/10; *UL*⁻, 0/16 (my unpublished results).

A similar mechanism could also explain the differences in transdetermination frequencies using different treatments (Table 2). According to a recent hypothesis¹⁰, transdetermination occurs when cells capable of it (Fig. 1b), by juxtaposition with cells having sufficiently different positional values, are stimulated to a certain critical amount of proliferation. In intact *UM*⁽⁻⁾ fragments, the fusion of the radial cut surfaces regularly confronts the cells capable of transdetermination with cells having nearly maximal disparities in circumferential values. The amount of intercalary growth thus induced would often trigger transdetermination. In pure *UM*⁻ reagggregates, however, the chance for the transdetermination-competent cells to confront cells with positional disparities large enough for the stimulation of transdetermination is very small. In intact *UL*⁽⁻⁾ fragments and in pure *UL*⁻ reagggregates, the cells capable of transdetermination simply could never meet cells with a positional disparity sufficient for the stimulation of transdetermination, because of the low density of circumferential values in this disk region^{3,6}. In mixed *UM*⁽⁻⁾ and *UL*⁽⁻⁾ reagggregates, however, juxtaposition to 'feeding layer' cells with large disparities in positional values would often cause a sufficient amount of proliferation in the cells capable of transdetermination to stimulate them to undergo this change. Earlier reports of sharply increased transdetermination frequencies in imaginal disk reagggregates^{16,17} can similarly be explained by cell interactions of this kind, without having to assume a specific effect of the dissociation procedure *per se* ('wounding effect') on transdetermination.

I thank Suzanne Glenn and Becky Hsei for technical assistance. I was supported by a fellowship from the Swiss National Science Foundation and by a grant from the United States National Institutes of Health.

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Received 18 June; accepted 22 August 1977.

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Haemopoietic stem cells of rats but not of mice express Th-1.1 alloantigen

IN mice thymus-derived lymphocytes express θ -antigen specificities in two allelic forms, Th-1.1 (formerly called θ -AKR) and Th-1.2 (θ -C3H), which are distributed reciprocally among the various mouse strains^{1,2}. Rats have also been tested for the occurrence of antigens of the Th-1 system, but so far all strains have been positive with anti-Th-1.1 only^{3,4}. Rats also differ from mice in the concentration of Th-1.1 carrying cells, which, in rats, is much higher in the bone marrow and lower in the peripheral lymphoid organs⁵. The stage at which haemopoietic stem cells differentiate towards thymocytes carrying the θ alloantigen is still ill-defined. We present here evidence that in rats Th-1.1 is already expressed on haemopoietic stem cells while murine haemopoietic stem cells have not yet developed this θ alloantigen.

Haemopoietic stem cells are defined operationally by their capacity to form cell colonies⁶, proliferate in diffusion chambers⁷ and, of course, repopulate a conditioned bone marrow recipient. The latter activity can be inhibited by sensitising the prospective bone-marrow recipient against antigens occurring on the donor's stem cells or by incubating the donor's marrow with antibodies against his stem cells before transferring it to the recipient. Both experimental models served as indicator systems for Th-1 antigens on stem cells in the following experiments.

Mouse strains CBA, C3H, (C57BL/6 × CBA-T6)F₁, A and BALB/c, all Th-1.2 and AKR (Th-1.1) were obtained from The Jackson Laboratory, Bar Harbor, Maine and AKR/Cum (Th-1.2) mice from Cumberland View Farms, Clinton, Tennessee. The two AKR sublines are antigenically similar with respect to markers Ly-1, Ly-2, Ly-3, TL, H-2, G_{1x} and Gross cell surface antigen, while differing for Thy-1⁸. A/0 AKR (Th-1.1) mice were produced from mice originally supplied by Dr E. A. Boyse of the Sloan-Kettering Institute, New York. They are congenic to A/J and differ only at the Th-1 locus. Inbred Lewis rats were produced in our laboratory: 90% of their thymocytes were lysed by anti-Th-1.1 but not by anti-Th-1.2 in the microcytotoxicity test. These antisera had a cytotoxic titre of 1:512 and more and had been produced by

Table 1 Transplantation of bone marrow from Th-1.1 mice or rats to Th-1.2 mice presensitised against Th-1.1

Expt no.	Recipient* Th-1.2	Immunisation† Th-1.1	Th-1.2	Take (AKR/J) Th-1.1	8	Chimaerism on days‡ 14			
1	(C57BL/6 × CBA-T6)F1	A/θAKR		+	50/0	18/2	48/2	50/0	20/0
2	(C57BL/6 × CBA-T6)F1	Lewis		+	15/5	18/2	98/2	50/0	0/20
				Take (Lewis)					
3	(C57BL/6 × CBA-T6)F1	AKR/J		—	20/0	15/2	13/0	20/0	20/0
4	(C57BL/6 × CBA-T6)F1		AKR/Cum	+	0/2	0/100	2/98	2/98	
5	CBA	AKR/J		—	10/0	66/45	14/0	50/0	8/2
6	CBA		C ₃ H	+	45/5	20/30	12/88	10/90	0/50
7	AKR/Cum	AKR/J		—			20/0	17/2	50/0
8	AKR/Cum		BALB/c	+			13/87	0/100	0/100

*Conditioned with 750–900 rad ¹³⁷Cs, 131 rad per min 24 h before transplantation of 2 × 10⁷ AKR/J mouse or 10⁸ Lewis rat bone marrow cells.

†10⁸ thymocytes were injected i.p. three times 5 d apart.

‡Allogeneic chimaeras: no. without/no. with the T6 marker chromosome; xenogeneic chimaeras: no. of alkaline negative mouse granulocytes/no. of alkaline positive rat granulocytes.

repeated immunisation of CBA, C₃H, AKR/J mice or Lewis rats with Th-1 incompatible thymocytes (10⁸ cells injected three times 5 d apart).

Failure of engraftment due to presensitisation against antigens on donor-type haemopoietic cells is a very sensitive indicator system. It reveals also minor antigen incompatibilities where serological *in vitro* tests fail⁹. To avoid any undesired cross-immunisation of the recipient from antigens on the donor's thymocytes other than Th-1.1, bone marrow recipients were immunised with thymocytes of third-party strains sharing the Th-1.1 antigen of the donor strain but no other tissue antigens which might prevent haemopoietic engraftment. Intraspecies sensitisation was therefore combined with interspecies bone marrow transplantation and vice versa. Interspecies xenogeneic bone marrow transplantation from rat to mouse (for conditions see Table 1 legend) regularly leads to complete chimaerism¹⁶. The following experimental design was used: prospective bone marrow Th-1.2 recipient mice were sensitised against Th-1.1 or Th-1.2 thymocytes of rats or different mouse strains. They were subsequently irradiated and transfused with third-party marrow from AKR/J or Lewis rats (both Th-1.1). Failure of engraftment was taken as evidence for sensitisation against alloantigens shared by sensitising cells and haemopoietic stem cells of the donor strain. Chimaerism was tested in the femora by chromosomal analysis in allogeneic chimaeras using the T6 marker chromosome and by selective alkaline phosphatase staining of rat granulocytes¹⁵ in xenogeneic chimaeras.

Experiments 1 and 2 in Table 1 show that immunisation with Th-1.1 thymocytes of A/θ AKR mice or Lewis rats did not interfere with a take of subsequently transferred AKR/J bone marrow. Control series of untransplanted mice of the recipients' strains receiving AKR/J or Lewis or A/θ-AKR thymocytes had regularly shown high titres (< 1:256). Rat bone marrow, in contrast, failed to grow in mice sensitised against the mouse Th-1.1 antigen. That this failure of engraftment is not inherent to the xenogeneic donor-recipient combination itself is shown in experiments 4, 6 and 8 where rat marrow grew easily in mice sensitised against thymocytes of congenic or allogeneic Th-1.2 strains.

Table 2 Chimaerism in mice after transfer of rat marrow pre-incubated with anti-Th-1.1 or anti-Th-1.2 serum

Incubation* with	Chimaerism on day 14†
Anti-Th-1.2 (AKR/J-anti-C ₃ H)	10/60, 5/95, 1/18, 0/10
Anti-Th-1.1 (C ₃ H-anti-AKR/J)	5/1, 4/0, 0/0, 0/0, 20/3, 20/0, 18/0
Normal mouse serum	0/100, 0/20, 3/0, 0/60, 0/100, 0/100, 2/98, 0/0
No serum	0/50, 0/3, 0/30, 3/17

*0.25 ml for 30 min at 37 °C.

†No. of alkaline negative mouse granulocytes per no. of alkaline positive rat granulocytes.

Chimaerism was an all-or-none phenomenon which occurred uniformly in all animals of an experimental group.

Group-specific stem cell toxicity of anti-Th-1.1 was also demonstrated by incubating rat marrow with anti-Th-1.2 (AKR/J-anti-C₃H) and anti-Th-1.1 (C₃H-anti-AKR/J) serum. Table 2 shows that only the latter antiserum prevented a take of the rat marrow, which was suggested by the absence of circulating rat granulocytes and proved by a completely aplastic bone marrow histology (Hoffmann-Fezer, unpublished) of the recipient mice. In contrast, transplantation of allogeneic bone marrow from AKR/J mice to (C57BL/6 × CBA-T₆)F₁ recipients was not inhibited by a pre-incubation of anti-Th-1.1 (CBA-anti-Lewis) serum (not shown in Table 2) though this antiserum reacted strongly with AKR/J (1:1024). The transfer experiments clearly show that sensitisation against Th-1.1 positive rat marrow either *in vivo* by immunisation of the marrow recipient against Th-1.1 mouse marrow or *in vitro* by incubation of the donor marrow with anti-Th-1.1, inhibits the repopulating capacity of haemopoietic rat stem cells. The immunogenetic specificity of this inhibition is evident because sensitisation against Th-1.2 in otherwise identical conditions did not inhibit haemopoietic repopulation of mice with rat marrow. It is difficult to preclude that a transplantation antigen closely linked to Th-1.1 rather than the θ antigen itself occurs on the rat's haemopoietic stem cells and prevents repopulation implying a tissue antigen occurring in rats and AKR/J but absent from AKR/Cum and the other Th-1.2 mouse strains tested: this seems unlikely, however.

On the other hand, the allogeneic cell transfer experiments also show unequivocally an absence of the Th-1.1 antigen on mouse haemopoietic stem cells. Stem cells of the Th-1.1 donors preserved the capacity to induce haemopoiesis in recipients in spite of an antiserum treatment inhibiting the Th-1.1 lymphocytes present together with the stem cells in the donor cell inoculum. That these stem cell-sparing anti-θ antibodies were active against the donor strain lymphocytes can be shown by their suppression of graft-versus-host reactions¹⁰.

The absence of the θ antigen from mouse stem cells is in agreement with reports on anti-mouse brain serum from rabbits, absorbed with B-cells, which spared haemopoietic stem cells and suppressed T cell-derived graft-versus-host disease when incubated with the donor's marrow¹¹. Anti-brain serum, however, does not lose its stem-cell toxicity after exhaustive absorption with T cells¹². Several other reports deal with antisera against haemopoietic or colony-forming stem cells. As a stem-cell label such antisera are, however, of little value because they usually react with species-specific antigens or H-2 alloantigens occurring on cells of various other organs.

Th-1.1 in rats is the first alloantigen restricted to stem cells and θ antigen-bearing cells. It is not only absent from B lymphocytes but, in contrast to mice, also from most peripheral T cells¹³. Again in contrast to mice where the concentration of Th-1.1 lymphocytes in the marrow is below 5%¹—Williams found 30–45% Th-1.1 positive cells in the rat bone marrow⁵. Considering the poor

immunocompetence of rat marrow Williams classifies Th-1.1 marrow cells as prothymic—having expressed their Th 1.1 molecule already in the bone marrow—rather than as mature post-thymic lymphocytes. Such a comparatively early expression of the Th 1.1 antigen in rat marrow may explain why Th 1.1, in the rat, can be detected also on haemopoietic stem cells. While this is speculative, the restrictive specificity of the Th-1.1 antigen, which is absent from myeloid cells and, in the rat, from over 90% of thoracic duct and lymph-node cells^{1,3}, should make it a useful marker of haemopoietic stem cells.

I thank Ms J. Molitor and B. Glöckner for technical assistance.

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Received 6 May; accepted 23 August 1977.

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Catecholamine hormone receptors are reduced on chronic lymphocytic leukaemic lymphocytes

ADENYLATE cyclase (EC 4.6.1.1.) is a bifunctional enzyme that responds to biological signals such as hormones as well as catalysing the synthesis of cyclic 3',5'-AMP. Several reports indicate that the hormonal responsiveness of this enzyme in transformed or neoplastic cells is altered¹⁻³. This may explain the low cyclic AMP levels that have been associated with the unregulated cellular proliferation seen in transformed cells⁴⁻⁶. There is also evidence to implicate cyclic AMP in normal lymphocyte activation^{7,8}. Comparison of circulating lymphocytes from chronic lymphocytic leukaemia (CLL) patients with those from normal human controls indicates that cyclic AMP levels^{9,10}, cyclic nucleotide phosphodiesterase^{11,12} and adenylate cyclase¹³ activities are changed in the CLL lymphocyte. The membrane enzyme activity of 5' nucleotidase¹⁴ as well as complement¹⁵, antigen¹⁶ and lectin¹⁷ binding are also altered in the CLL plasma membrane. The observation that catecholamine hormone (β -adrenergic) responsiveness is depressed in CLL lymphocytes is further evidence for a functionally altered plasma membrane¹³. Until now, it has not been possible to identify the molecular lesion responsible for the reduced catecholamine sensitivity. But, with the availability¹⁸⁻²⁰ of radiolabelled molecules that bind efficiently and directly to specific β -adrenergic hormone receptor sites (for example (–)³H-dihydroalprenolol), we can now determine whether the CLL adenylate cyclase defect occurs in the β -adrenergic hormone-receptor complex, in the transducing mechanism that couples the receptor to the catalytic component, or in the intrinsic catalytic mechanism. We show here that the number of β -adrenergic hormone receptor sites is reduced on CLL lymphocyte membranes while the catalytic capacity of the cyclase enzyme is normal. The low density of catecholamine hormone receptors could account for the altered cyclic AMP metabolism and may

contribute to the unregulated growth of CLL lymphocytes.

Lymphocytes were isolated from heparinised blood from healthy human volunteers (100–400 ml) and from patients with chronic lymphocytic leukaemia (10–30 ml). The diagnosis of CLL was made after complete haematological evaluation including bone marrow biopsy. All steps in the isolation were done using sterile techniques. The lymphocytes were separated by centrifuging the blood on Ficoll-Hypaque density gradients using the method of Boyum²¹. Cells at the interphase were collected and diluted 1:1 with 199 medium plus 5% foetal calf serum, centrifuged at 450g for 10 min, and the resulting pellet was washed with 20 ml of the same medium. The washed cells were further purified immediately or resuspended in 40 ml 199 medium plus 10 ml calf serum, placed in a Falcon 75 cm² flask and stored in a 37 °C, 5% CO₂ humidified incubator (up to 10 h).

Subsequently, the non-adherent mononuclear cells were collected by centrifugation at 200g for 10 min. The cells were resuspended to a concentration of about 5×10^7 cells per ml, and passed over nylon wool. Lymphocytes were collected by centrifugation (300g for 10 min) and washed twice (50 ml, 20 ml) with phosphate buffered saline pH 7.2 containing 0.1% gelatin. Greater than 90% of the cells were lymphocytes by microscopy. In selected experiments separation of B and T cells with recovery of both depleted and enriched fractions was performed as described by Kaplan *et al.*²². The final pellet was resuspended in 15 ml cold 50 mM Tris-HCl, 10 mM MgCl₂, pH 8.1 at 4 °C (incubation buffer), allowed to swell for 20 min, and homogenised by 25 up and down strokes in a pre-chilled (0 °C) Dounce homogeniser. The homogenate was centrifuged at 25,000g for 15 min, the pellet was rinsed once with cold incubation buffer (1 ml buffer per 5×10^7 cells). Adenylate cyclase activity of this membrane preparation was determined by the method of Brown²³. Protein was measured by the method of Lowry *et al.*²⁴.

The procedure for determining the density of hormone receptor sites on lymphocyte membranes was based on the method of Williams *et al.*²⁵. To tubes containing (–)³H-dihydroalprenolol (final concentration 1–80 nM) with and

Table 1 Adenylate cyclase activity of normal and CLL lymphocytes

Cell population	Adenylate cyclase activity (pmol per min per mg protein)		
	Basal	F ⁺ (10 mM)	Isoprenaline (10 μ M)
Normal lymphocytes	39	131	103
CLL lymphocytes	24	70	32

Adenylate cyclase activity was measured by analysis of the cyclic AMP²³ produced in a 10-min, 30 °C, incubation of the lymphocyte membranes in a buffer containing 40 mM Tris, 5 mM Mg²⁺, 5 μ g ml⁻¹ R020-1724 (Hoffman-La Roche), 1 mM dithiothreitol, 2 mM ATP and additives at the concentrations indicated. The values are averages of duplicates from a representative experiment.

without ($\pm$)propranolol (10^{-5} M final concentration) was added 160 μ l of lymphocyte homogenate (40–130 μ g protein) to give a total volume of 200 μ l. Tubes were incubated for 13 min at 37 °C. Incubation was terminated by adding 4 ml of ice-cold incubation buffer to the tube and rapidly filtering contents through a Gelman-type A/E glass fibre filter under low vacuum. The filter was rinsed (ice-cold incubation buffer) with a 4 ml wash of the tube and a final (8 ml) rinse. Filters were placed in 10 ml of 10% BioSolv (Beckman), 90% toluene, 0.4% omnifluor, scintillation cocktail and allowed to stand overnight before counting.

In each experiment, 'nonspecific' binding was determined by parallel assay tubes which contained a large excess (10 μ M) of ($\pm$)propranolol. 'Specific' binding was defined

as the difference between the total and nonspecific binding.

The (–)³H-dihydroalprenolol was from New England Nuclear who prepared the radiolabelled product by catalytic reduction of (–)alprenolol using tritium gas with palladium as a catalyst. The molecular structure (determined by mass spectroscopy) is dihydroalprenolol and the tritium is probably found at the unsaturated bond in the aliphatic chain on position 2 of the aromatic ring.

The reactivity of the CLL lymphocyte adenylate cyclase to the β -adrenergic agonist, (–)isoprenaline (Sigma) is appreciably less than that of the enzyme prepared from normal lymphocytes (Table 1). However, fluoride stimulation of the CLL enzyme is comparable with that of normal controls (threefold stimulation). This response of the enzyme to fluoride indicates that the catalytic mechanism of the CLL enzyme is functioning properly.

Using (–)³H-dihydroalprenolol as a specific β -adrenergic ligand to bind directly and specifically to β -adrenergic hormone receptors, we compared crude membrane preparations from normal and CLL lymphocytes. Figure 1 illustrates the reduced binding of the radiolabelled ligand by CLL membranes. The normal cells showed considerable binding which corresponded to approximately 6,000 molecules bound per cell. Ligand binding to CLL membranes was too low to assess accurately, but it was less than 2,000 molecules per cell. The control dose-response curve exhibits saturation at 6×10^{-8} M, indicating an approximate K_d of 3×10^{-8} M for the dihydroalprenolol. Both control and CLL binding reactions showed about 70% specific binding of the (–)³H-dihydroalprenolol in the experimental conditions. In addition to the saturability and high affinity, the specificity of the dihydroalprenolol binding was indicated by its stereospecificity (1-stereoisomers of isoprenaline and propranolol were more efficient in displacing the bound (–)³H-dihydroalprenolol than the *d*-stereoisomers) and the binding

showed rapid kinetics for association reaching a steady-state within two minutes. Furthermore, the displacement of (–)³H-dihydroalprenolol by 10^{-6} M propranolol was approximately the same as 10^{-4} M isoprenaline which is consistent with the potency of these agents on other well characterised β -adrenergic receptors.

All of the CLL patients in this study had increased percentages of lymphocytes with monoclonal surface immunoglobulin (Ig) suggesting that the disease probably represents a proliferation of B lymphocytes¹⁶. To exclude the possibility that the depressed (–)³H-dihydroalprenolol binding was not simply a characteristic of cells with surface Ig, separation of normal lymphocytes into T and B populations was performed. Table 2 shows, in fact, that membranes isolated

Table 2 (–)³H-Dihydroalprenolol bound to human lymphocytes

Cell population	(–) ³ H-dihydroalprenolol bound† (fmol per mg membrane protein)
Normal lymphocytes (8)*	682 ± 114
'T' lymphocytes, normal (2)	532 ± 132
'B' lymphocytes, normal (2)	1,439 ± 83
CLL lymphocytes (10)	220 ± 69

Lymphocyte preparation and separation into 'B' and 'T' subpopulations were performed as described in the text. Of the cells in the 'B' fraction, 61% have surface immunoglobulin, while 89% of the 'T' rich portion formed red blood rosettes²². Binding was measured²⁵ at a concentration of 5×10^{-8} M (–)³H-dihydroalprenolol. The averages of triplicate determinations were done for each experiment and the averages used to determine the standard error of the differences between the normal and CLL lymphocytes. Normal and CLL values showed a significant statistical difference which is represented by $P < 0.001$.

*Value in parentheses is number of separate experiments.

† Mean ± s.e.m.

from non-T cells (surface Ig positive) bind more (–)³H-dihydroalprenolol than those isolated from T lymphocytes. This indicates that enrichment of B cells in the CLL lymphocyte population could not account for the observed hormone receptor differences between normal and CLL cells.

CLL lymphocyte adenylate cyclase is β -adrenergic unresponsive, although the homogenates exhibit fluoride-stimulated adenylate cyclase activity, showing that the catalytic component of the enzyme is behaving normally. We have shown that the density of the β -adrenergic hormone receptor site is decreased in CLL lymphocytes. This alteration in the number of β -adrenergic hormone receptor sites could explain the loss of CLL β -adrenergic adenylate cyclase responsiveness observed first by Polgar *et al.*¹³. It may also account for the depressed cellular levels of cyclic AMP found in CLL lymphocytes⁹. Depressed cyclic AMP levels are known to be correlated with unregulated cellular growth⁴, a characteristic of the neoplastic state.

A decreased capacity of cultured transformed cells to bind radiolabelled hormones has been recently observed. Differences in polypeptide hormone receptor sites^{26,27} as well as the catecholamine hormone receptors²⁸ were found in comparisons of virus-transformed cells with normal, growth regulated cells. Previous studies of 5' nucleotidase¹⁴ complement¹⁵ antigens¹⁶ and plant lectins¹⁷ have all indicated that the membrane of the CLL cells is structurally and functionally aberrant. Our observations show that the density of β -adrenergic hormone receptor sites is decreased on the CLL lymphocyte surface. Membrane alterations such as these that interfere with basic mechanisms of biological communication may be fundamental to the aetiology of diseases characterised by unregulated cellular proliferation.

We thank Elizabeth Anton for technical assistance. The separation of lymphocyte subpopulations was performed in the laboratory of Dr M. Kaplan. This research was supported by grants from the USPHS, the Veterans'

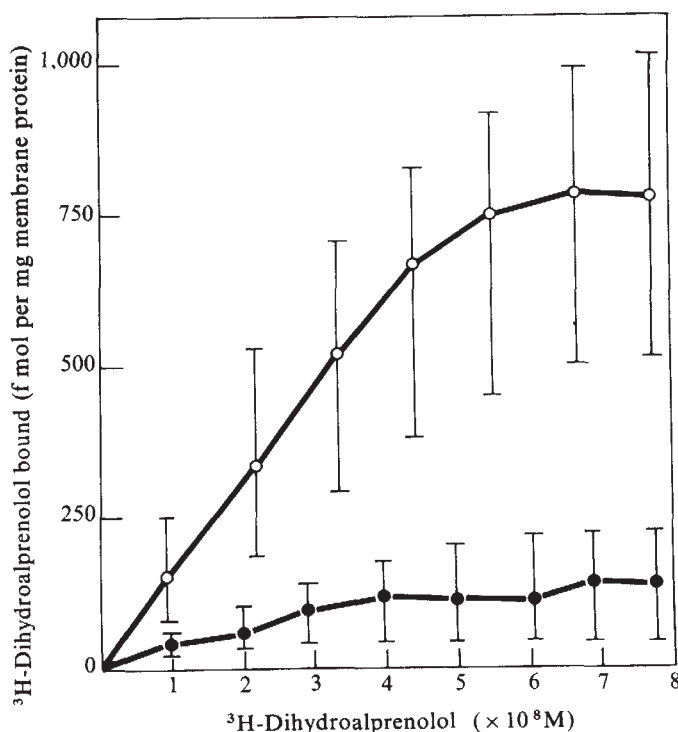


Fig. 1 (–)³H-dihydroalprenolol binding, as a function of concentration, was measured for lymphocyte membranes prepared as described in the text. This figure shows a single representative experiment with the range bars enclosing the area of all the experiments performed (12 normal and 10 CLL lymphocyte preparations). ○, Normal lymphocytes; ●, CLL lymphocytes.

Administration, the Minnesota Medical Foundation, the Graduate School of the University of Minnesota, and the Leukaemia Task Force.

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Received 21 April; accepted 8 August 1977.

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Actin co-caps with concanavalin A receptors

PREVIOUS studies have given mainly indirect^{1,2}, and some direct^{3,4} evidence that the mobility of surface membrane receptors may be controlled by cytoplasmic microfilaments and microtubules. It has been proposed^{1,2} that the receptor–cytoskeletal complex may have important roles in cell–cell recognition, cell motility and cell division. This study, using two fluorochromes to identify concanavalin A (con A) receptors and cytoplasmic actin in the same cell, shows that con A binding to neoplastic and embryonic cell surfaces results in dissolution of actin filaments and that con A receptors and actin co-cap and occur in closely related sites in untreated and cytochalasin B-treated cells.

Monolayer cultures of rat fibroblasts were prepared from normal adult kidney, dimethylnitrosoamine (DMN)-induced renal mesenchymal tumours⁵, transformed kidney cell lines derived from DMN-treated rats⁶, 15–20 d foetal kidney or lung⁷ and 5–10 d whole embryos. The cells were tested either untreated, or after exposure for 20–60 min to 2×10^{-5} M cytochalasin B, 10^{-2} to 10^{-6} M colchicine, 10^{-5} M vinblastine, 2×10^{-3} M procaine or $10 \mu\text{g ml}^{-1}$ trypsin.

Marginal and/or random surface staining was seen in adult, tumour, transformed, embryonic and foetal fibroblasts incubated with fluorescein–isothiocyanate (FITC)-labelled con A at 4 °C, and in adult and foetal fibroblasts incubated at 37 °C (Fig. 1a, Table 1). In contrast, tumour, transformed and embryonic fibroblasts, incubated with FITC-con A at 37 °C for 20 min, showed staining in fine clusters (Fig. 2a), and at the cell margins and processes. Incubation of these cells for increasing time periods

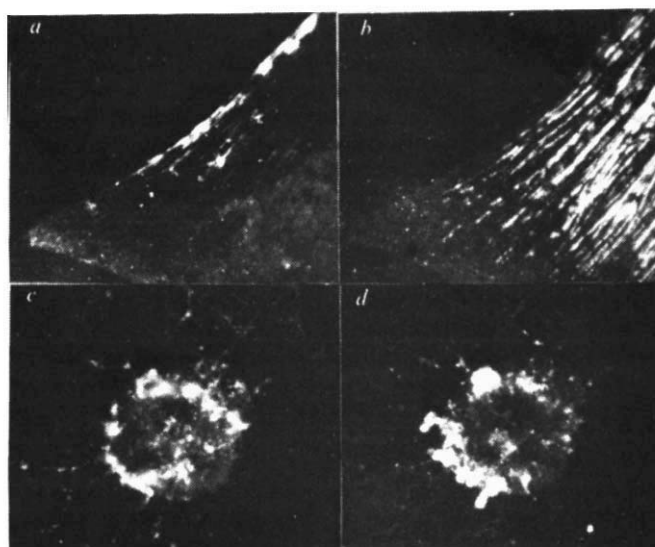


Fig. 1 FITC-con A followed by AAA/R-AHG staining of cultured adult kidney fibroblasts. Con A receptors were demonstrated by immunofluorescence reactivity^{1,5} of cell surfaces with FITC-labelled con A. Cytoplasmic actin was detected by AAA traced with R-AHG. The fluorescein: protein molar ratio and protein content of the conjugates were 4.9 and $300 \mu\text{g ml}^{-1}$ for FITC-con A, and 3.3 and 4 mg ml^{-1} for R-AHG. Tests were done at 4 °C or at 4 °C warmed to 37 °C for 20–360 min. Specificity of the FITC-con A staining was established by inhibition with unlabelled con A ($1,000 \mu\text{g ml}^{-1}$) or with 0.1 M α -methyl-D-mannopyranoside. The AAA specificity was established by immunoabsorption with muscle actin⁸. *a*, Adult kidney fibroblast, sub-1 stained with FITC-con A showing linear staining of the cell periphery ($\times 330$). *b*, Same cell as in (*a*) stained with AAA/R-AHG showing parallel actin filaments located in closely related sites as those seen in (*a*) ($\times 330$). *c*, Adult kidney fibroblast, sub-1, treated with cytochalasin B at 37 °C for 20 min and stained with FITC-con A at 37 °C for 20 min showing coarse clusters in the cell periphery ($\times 330$). *d*, Same cell as in (*c*) stained with AAA/R-AHG showing coarse clusters in sites closely related to those seen in (*c*). ($\times 330$.)

produced fine to coarse clusters in a central mass with clearing of the cell periphery at 40 min, a supranuclear cap of clusters at 60 min (Fig. 2c) and perinuclear globules at 120 min persisting until 360 min. Cells pretreated with 1% or 4% paraformaldehyde at 4 °C or 37 °C for 10 min showed staining restricted to cell margins both at 4 °C and 37 °C.

After 20–40 min of cytochalasin B (BL) treatment, adult and foetal fibroblasts tested with FITC-con A at 37 °C, showed fine clusters in cells with preserved cell shape and in contracted cells with cytoplasmic arborisations. After treatment for 40–60 min, the cells showed coarse, peripheral clusters in incompletely (Fig. 1c) or completely rounded up cells; supranuclear caps and perinuclear globules were not seen. Procaine treatment also produced fine to coarse clusters but colchicine and vinblastine did not alter the marginal and/or random surface staining seen in untreated cells. Trypsin induced a staining pattern of fine clusters. The cytoskeletal-disrupting drugs did not cause any changes in the initial staining pattern of fine clusters seen in tumour, transformed and embryonic cells (Table 1) but, with increasing incubation times, supranuclear caps and perinuclear globules were not seen.

Anti-actin antibody (AAA) (ref. 8) staining, traced with a rhodamine-labelled goat anti-human (γ) globulin (R-AHG), showed long parallel bundles of thick filaments in adult, embryonic and foetal fibroblasts (Fig. 1b) and short thin filaments with occasional fine aggregates in tumour and transformed cells⁹. Previous incubation of tumour, transformed, embryonic or foetal cells with FITC-labelled or unlabelled con A for 20 min and subsequent testing with AAA showed staining of fine aggregates, diffuse staining or no staining (Fig. 2b). The changes in staining patterns were most marked in neoplastic cells (> 90% cells), moderate in embryonic cells (70–90%) and minimal in foetal cells (< 10%). The change was more pronounced at 4 °C than at 37 °C. It was not seen in adult fibroblasts or in cells pre-treated with

Table 1 Immunofluorescence staining of monolayer cultures of adult, neoplastic, embryonic and foetal fibroblasts by FITC-con A or AAA

Experimental conditions	Adult, foetal kidney or lung fibroblasts		Tumour, transformed or whole embryo fibroblasts	
	FITC-con A	AAA	FITC-con A	AAA*
4 °C	Cell margins	Filaments	Cell margins and random	Filaments and fine aggregates
37 °C	Cell margins	Filaments	Fine clusters, supranuclear caps or perinuclear globules	Filaments and fine aggregates
Cytochalasin B†	Fine or coarse clusters	Fine or coarse aggregates	Fine clusters	Fine or coarse aggregates
Procaine	Fine or coarse clusters	Fine or coarse aggregates	Fine clusters	Fine or coarse aggregates
Colchicine or vinblastine	Cell margins	Filaments	Fine clusters	Filaments and fine aggregates
Trypsin	Fine clusters	Fine aggregates, diffuse or negative	Fine clusters	Fine aggregates, diffuse or negative

Viable monolayer cultures were treated for 20–360 min with $300 \mu\text{g ml}^{-1}$ FITC-con A in tissue culture medium 199. Drug-treated cells were pre-incubated with the reagent for 20–60 min followed by a further period of incubation, with or without the reagent, with FITC-con A for 20–120 min. AAA staining was carried out with cultures, fixed by air-drying and leaving at 4 °C for 2 h. Staining was still detectable with $20 \mu\text{g ml}^{-1}$ FITC-con A or 1:256 dilution AAA. The staining patterns were present in > 90% of cultured cells.

*Previous treatment of viable cultures with FITC-labelled or unlabelled con A resulted in dissolution of actin filaments.

†Treatment of control cultures with the cytochalasin B solvent, dimethylsulphoxide, had no effect on FITC-con A or AAA staining patterns.

FITC-labelled AHG at 4 °C or 37 °C, or pre-cooled at 4 °C for 10 min and tested at 4 °C with AAA.

Our data show that con A receptors may be structurally linked to actin since the two components occur in closely related sites in all the cell types examined (Figs 1, 2). This link may take the form of an actin sub-set, not stained by AAA, which restricts con A receptor mobility in adult and mature foetal cells and which is poorly expressed in neoplastic and embryonic cells. The suggestion is

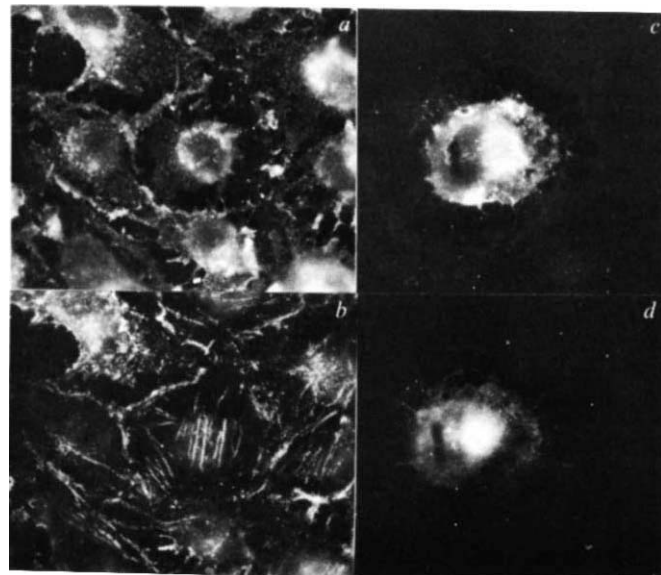


Fig. 2 FITC-con A followed by AAA/R-AHG staining of cultured embryonic or tumour fibroblasts. *a*, Tumour fibroblasts, GHF117BL, sub 88 reacted with FITC-con A at 37 °C for 20 min showing fine clusters. ($\times 330$) *b*, Identical field as (*a*) stained with AAA/R-AHG at 37 °C showing filamentous and diffuse or aggregate staining. Note that areas stained in an aggregated or diffuse pattern occur in areas similar to those of FITC-stained clusters shown in (*a*), but filaments bear no relationship to these clusters. ($\times 330$) *c*, Embryonic fibroblast, sub-1, reacted with FITC-con A at 37 °C for 60 min showing a diffusely stained supranuclear cap containing fine clusters. ($\times 330$) *d*, Same cell as in (*c*) reacted with AAA/R-AHG showing a cap located in a similar position and stained in a similar pattern as in (*c*). ($\times 330$)

supported by our finding that cluster formation may occur in cells containing actin filaments (Fig. 2*a, b*) and can be induced in adult or foetal cells by cytochalasin B treatment. The proposal is also consistent with the observed decrease in membrane-associated actin in tumour cells¹⁰. Because the linear marginal distribution of con A receptors is in close proximity to actin filaments (Fig. 1*a, b*) we suggest that the actin sub-set may anchor con A receptors to actin filaments. An actin sub-set is also thought to limit con A receptor mobility in normal alveolar macrophages¹¹. Lattice microfilaments¹² are a possible candidate for this actin sub-set.

Con A binding to surface receptors result in conversion of filamentous actin to aggregates or a diffusely stained form. These latter forms of actin seem essential for capping and endocytosis since FITC-con A and AAA-stained areas occur in close proximity (Fig. 2*c, d*) and the processes are inhibited by CB. Microtubules may also have a role in capping and endocytosis as these processes are inhibited by colchicine or vinblastine. Inhibition of capping by cytochalasin B, vinblastine, colchicine or procaine supports the results of previous workers^{13,14}. The cytochalasin B-induced 'caps' reported in a recent study³ may not represent true caps but may result from cytochalasin B-induced disruption of actin filaments.

This study was supported by grants from the Anti-Cancer Council of Victoria and the National Health and Medical Research Council. We thank Vivien Randell, Helen King and John Lee for technical assistance, Dr C. R. Lucas of the Fairfield Infectious Diseases Hospital for AAA serum and Dr H. A. Ward and staff for preparation of the fluorescent conjugates.

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Received 5 July; accepted 10 August 1977.

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Actin and tubulin co-cap with surface immunoglobulins in mouse B lymphocytes

CYTOSKELETAL control of cell surface receptor movements¹ has been suggested by several authors^{2,3} and is compatible with the fluid mosaic model of cell membranes⁴. Evidence for this hypothesis comes mostly from experiments using drugs which alter the activity of cytoskeletal components^{3,5,6}. We report here co-capping of actin and tubulin with immunoglobulins (Ig) in B lymphocytes from mouse spleen. These findings provide structural evidence for cytoskeletal control of receptors.

Purified anti-actin and anti-tubulin antibodies were obtained respectively from the serum from a patient with chronic aggressive hepatitis, which contained specific anti-actin antibodies^{7,8} and from the sera of rabbits repeatedly immunised with pig brain tubulin⁹ adsorbed to alum (A.Z. and G.G., in preparation). These sera were passed through columns of Sepharose covalently linked¹⁰ respectively with rabbit skeletal muscle actin¹¹ or with pig brain tubulin¹⁴, and the absorbed antibody was eluted at pH 2.7. The specificity of these antibodies was shown by immunodiffusion—a single precipitation line was observed against the antigen (Fig. 1)—by immunoelectrophoresis, and by immunofluorescence. In the immunofluorescence test the anti-actin antibody (AAA) was fixed on striated (I bands) and smooth muscle tissue sections and the anti-tubulin antibody (ATA) on mitotic spindles, cultivated fibroblasts and spermatocytes; cell staining was abolished by previous incubation of the antibody solution with actin and tubulin respectively.

Mouse spleen lymphocytes were incubated with rabbit antiserum against mouse Ig (RAMIG), either conjugated with rhodamine¹² or unconjugated, and then with sheep antiserum against rabbit Ig (SARIG) conjugated with rhodamine¹²; conditions were as described elsewhere¹³ and incubations were for 30 min at 0 or 37 °C. After this incubation the cells were suspended in 0.035 M citrate¹⁴, a drop of this suspension was applied on a slide and dried. For actin staining, cells

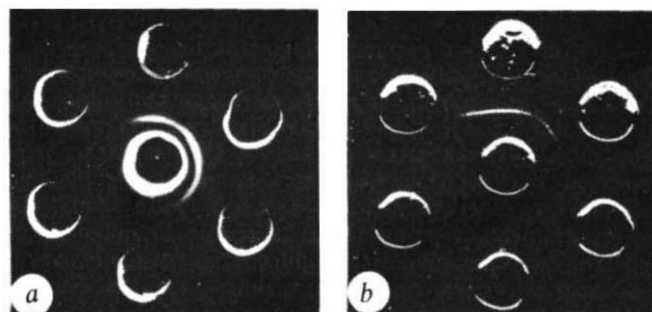


Fig. 1 Immunodiffusion experiments using the Ouchterlony technique. Readings were made between 24 and 48 h after a single filling of the wells. *a*, AAA serum was placed in the centre against decreasing concentrations of actin (3.0; 1.5; 0.75; 0.375; 0.19 mg ml⁻¹). To keep actin depolymerised, Agarose (Behring-Werke) was dissolved in 2 mM Tris-HCl buffer at pH 8.0 containing 0.2 mM ATP, 0.5 mM 2-mercaptoethanol and 0.2 mM CaCl₂ (ref. 10). *b*, ATA serum was placed in the centre against similarly decreasing concentrations of tubulin. Agarose was dissolved in 0.15 M NaCl containing 0.5 mM 2-mercaptoethanol. A single precipitation line is visible in both cases.

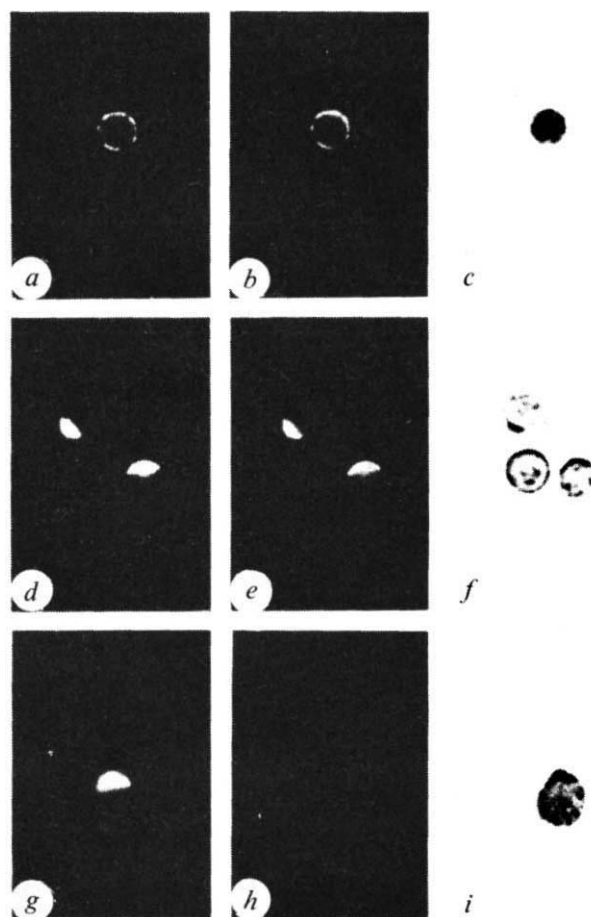


Fig. 2 Immunofluorescent staining of splenic B lymphocytes incubated in suspension with RAMIG and SARIG-rhodamine, then fixed with ethanol and incubated with AAA followed by anti-human IgG-fluorescein. All photographs were taken with a Zeiss ultraviolet photomicroscope equipped with epi-illumination and specific filters for rhodamine and fluorescein, using a Plan Apo-Chromate 63x/1.0 objective on HP5 Ilford black and white film. *a-c*, Incubation in suspension at 4 °C; *a*, rhodamine; *b*, fluorescein; *c*, phase contrast. *d-f*, Incubation in suspension at 37 °C; *d*, rhodamine; *e*, fluorescein; *f*, phase contrast. *g-i*, Incubation in suspension at 37 °C; as control, after ethanol fixation cells were incubated with human Ig instead of AAA and then with anti-human IgG. *g*, Rhodamine; *h*, fluorescein; *i*, phase contrast ($\times 2,000$).

were fixed for 30 s in absolute ethanol, incubated for 15 min with purified AAA and for a further 15 min with fluorescein conjugated Ig fraction of goat antiserum to human IgG (Miles Seravac). To avoid cross reactions between these antisera, the goat anti-Ig was passed on a solid immunoabsorbent made with glutaraldehyde-coupled mouse, rabbit and sheep Ig¹⁵. In control experiments the human purified AAA was replaced by a solution of normal human Ig with a similar concentration of proteins. For tubulin staining, cells incubated with RAMIG-rhodamine were fixed for 20 min in 3.7% formaldehyde and 5 min in acetone, and then incubated for 1 h with a solution of fluorescein conjugated¹³ rabbit purified ATA.

After incubation in suspension with RAMIG-rhodamine or RAMIG followed by SARIG-rhodamine, 70% of splenic lymphocytes had a positive staining. Cells incubated at 0 °C had a generally diffused labelling along the cell membrane with some patching and very rare capping, whereas a high proportion of cells incubated at 37 °C showed typical cap formation. When this incubation was followed by fixation of the cells and immunofluorescent staining for cytoskeletal proteins, the pattern of actin and tubulin corresponded to that of Ig (Figs 2 and 3). In non-capped cells, the fluorescence was weak, particularly for actin, and difficult to detect on the nuclear area. By contrast, in cells showing capped immunoglobulins,

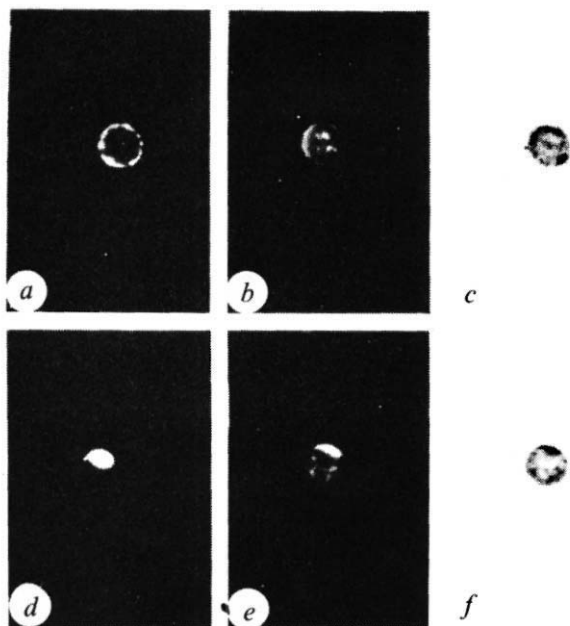


Fig. 3 Immunofluorescent staining of splenic B lymphocytes incubated in suspension with RAMIG-rhodamine then fixed with formaldehyde and acetone and incubated with ATA-fluorescein. *a-c*, Incubation in suspension at 4 °C; *a*, rhodamine; *b*, fluorescein; *c*, phase contrast. *d-f*, Incubation in suspension at 37 °C; *d*, rhodamine; *e*, fluorescein; *f*, phase contrast ($\times 2,000$).

the fluorescence for actin and tubulin was intense and restricted to the region of the cap. Among the cells which were rhodamine negative (that is, non-B cells) a high proportion (85%) were positive for actin and tubulin.

These findings show in a simple and well established model¹ that two cytoskeletal proteins, namely actin and tubulin, probably having different functions, move concurrently along the cell membrane with a single class of surface receptors—Ig. It has been reported that, in rabbit ovarian granulosa cells, concanavalin A-induced capping redistributes microtubules in the cytoplasmic region underlying the cap¹⁶. An analogous redistribution of surface antigens and actin has been described in Lu-106 cells after treatment with cytochalasin B¹⁷.

It is generally recognised that capping is dependent on cell metabolism^{1,18}. Several experiments have shown that cytochalasin B (a drug which alters microfilament morphology and function¹⁹) inhibits this phenomenon (though sometimes only partially)^{6,20} and hence it has been proposed that redistribution of cell surface molecules depends on microfilament activity. Microtubular integrity has also been shown to be linked with capping in B lymphocytes²¹. It has been proposed that microtubules and microfilaments exert skeletal and contractile actions respectively in regulating the movement of cell surface proteins³. Our findings are compatible with this hypothesis, and support the possibility that skeletal and contractile elements are linked to one another and/or to the same plasma membrane components.

This work was supported by the Swiss National Science Foundation (grant 3.692-0.76). We thank Miss M. Bouland for technical assistance and Mr J-C. Rumbeli and Mr E. Denking for photographic work.

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Intracellular binding sites for insulin are immunologically distinct from those on the plasma membrane

SPECIFIC high affinity binding sites for insulin have been shown to be located on the plasma membrane of target cells¹, and they are also present on purified intracellular organelles² including intact nuclei³, smooth and rough endoplasmic reticulum^{2,4}, and the Golgi apparatus⁵. Although these intracellular sites have certain binding characteristics similar to those on the plasma membrane^{2–5}, it is not known whether these binding sites share other biochemical features. Using an antiserum directed at the cell surface binding sites, we have found that several types of intracellular binding sites for insulin are immunologically distinct from those on the plasma membrane.

It has already been demonstrated that the sera of certain patients with extreme insulin resistance and acanthosis nigricans contain immunoglobulins directed against the plasma membrane binding site^{6–9}. When any of human monocytes or cultured lymphocytes, avian erythrocytes, or highly purified rat liver plasma membranes are preincubated with these antisera, the subsequent binding of radiolabelled insulin is inhibited^{6–9}. That these antibodies are specific for insulin binding sites on the plasma membrane is seen by their failure to inhibit both the binding of human growth hormone to intact human cultured lymphocytes^{6–9} and the binding of NSILA-s, an insulin-like peptide, to rat liver plasma membranes^{6–9}. Furthermore, using conditions where there is nearly complete inhibition of the specific binding of insulin, there is no inhibition of the binding of glucagon (Table 1). Other studies indicate that the binding of purified radiolabelled antibodies to plasma membranes is inhibited by insulin and insulin analogues in direct proportion to the biological activity of those analogues^{6–9}. These findings suggest that these antibodies bind either near to or at the portion of the plasma membrane that recognises and binds the insulin molecule.

When we pre-incubated rat liver plasma membranes with B2-1975 antiserum^{6–9} at a 1:100 dilution for 1 h at 4 °C, 85–90% of the subsequent binding of ¹²⁵I-insulin was inhibited (Table 1, Fig. 1). With progressive serial dilution, the inhibiting effect decreased; but even at a titre of 1:6,400, 25% inhibition of binding was demonstrable. In contrast to its ability to inhibit insulin binding to the plasma membrane, this antiserum had no significant effects on the binding of insulin to purified nuclei (Table 2). We have previously shown that the nuclear membrane is the major site of insulin binding to the cell nucleus¹². In contrast to the marked inhibition of the binding of insulin to plasma membranes, pre-incubation of nuclear membranes with B2-1975 antiserum had only small effects on insulin binding (Table 2, Fig. 1). Similar results were seen with the smooth endoplasmic reticulum (Table 2, Fig. 1). At a dilution of 1:100, specific binding of insulin to both nuclear membranes and smooth endoplasmic reticulum was inhibited by only 10–20%;

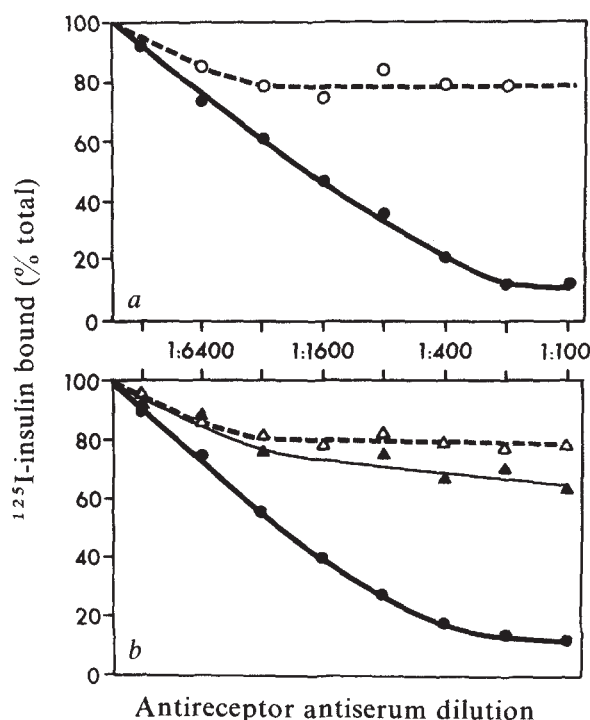


Fig. 1 Effects of serial dilutions of an anti-plasma membrane antiserum on ^{125}I -insulin binding to several types of cellular membranes. Plasma membranes¹⁰ (●) at 0.087 mg protein ml^{-1} (a) and 0.125 mg protein ml^{-1} (b), nuclear membranes¹³ (○) at 0.087 mg protein ml^{-1} , and smooth (△) and rough (▲) endoplasmic reticulum¹⁴ at 0.47 mg protein ml^{-1} were preincubated for 1 h with either normal human serum or antiserum at various dilutions as described in Table 1. ^{125}I -Insulin (0.5 ng ml^{-1}) and albumin (5 mg ml^{-1}) were then added and the incubation was continued for 2 h. Insulin bound to nuclear and plasma membranes was separated from free insulin by centrifugation in a microcentrifuge. Insulin bound to the rough and smooth endoplasmic reticulum was separated by a filtration through Oxoid filters (Amersham)^{3,11}. Each point is the mean of triplicate determinations. Binding is expressed as a percentage of that seen in the presence of pooled human serum. In the absence of antiserum specifically bound ^{125}I -insulin (% of total) was 3.95 ± 0.07 (a) and 5.75 ± 0.14 (b) for plasma membranes, 0.85 ± 0.008 for nuclear membranes, 3.4 ± 0.14 for smooth endoplasmic reticulum, and 1.9 ± 0.04 for rough endoplasmic reticulum. Values are the mean $\pm$ standard deviation.

the degree of inhibition, moreover, did not change with dilution of the antiserum up to 1:3,200.

The ability of this antibody to inhibit insulin binding to the rough endoplasmic reticulum was also much less than that seen with plasma membranes (Table 2, Fig. 1). At a dilution of 1:100, approximately one-third of the specific binding of insulin was inhibited. In contrast to the results obtained with smooth endoplasmic reticulum and nuclear membranes, the inhibition of insulin binding to the rough endoplasmic reticulum progressively diminished on further dilution of the antiserum.

There are two possible reasons why this antiserum partially inhibits insulin binding to intracellular membranes. In the case of the rough endoplasmic reticulum, it is possible that the inhibition observed represents the interaction of the antibody to plasma membrane insulin binding sites with nascent plasma membrane binding sites present on ribosomes. Since this antiserum (B2-1975) also contains antibodies directed against a variety of cell proteins⁹, it is possible that the non-progressive inhibition observed with nuclear membranes or smooth endoplasmic reticulum is caused by a second antibody present in the antiserum that only weakly inhibits binding. In either case, the present studies suggest that the insulin binding sites on these intracellular membranes from rat liver are

Table 1 Effect of an anti-plasma membrane antiserum on the binding of ^{125}I -insulin and ^{125}I -glucagon to rat liver plasma membranes

	^{125}I -hormone bound (% per 0.1 mg protein per ml)	
	Insulin	Glucagon
Control serum	4.0 ± 0.21	10.2 ± 0.13
Antiserum	0.5 ± 0.12	9.9 ± 0.40

Rat liver plasma membranes¹⁰ at 0.057 mg protein ml^{-1} were preincubated for 1 h at 4 °C with a 1:100 dilution of antiserum (B2-1975) (refs 6–9) or control serum in buffer containing 0.25 M sucrose, 10 mM MgCl_2 , 2 mM EDTA, 20 mM Tris (pH 7.85). After this pre-incubation, either ^{125}I -insulin³ at 0.75 ng ml^{-1} in the presence or absence of 200 $\mu\text{g ml}^{-1}$ of unlabelled insulin (to determine nonspecific binding) or ^{125}I -glucagon¹¹ at 0.5 ng ml^{-1} in the presence or absence of 50 $\mu\text{g ml}^{-1}$ of unlabelled glucagon was added with bovine serum albumin at 5 mg ml^{-1} . The incubation was continued at 24 °C for 2 h for insulin or 1 h for glucagon. Bound and free hormones were separated by centrifugation in a microcentrifuge^{3,11}. Each value is the mean $\pm$ the standard deviation for triplicate determinations. Nonspecific binding has been subtracted.

immunologically distinct from those found on the plasma membrane.

It is evident that, per unit of protein, plasma membranes bind more insulin than do the intracellular membranes (Table 2). Our preliminary studies suggest that this increased binding of insulin to plasma membranes is due to the higher affinity of its insulin binding sites. In contrast, the total number of insulin binding sites per unit of protein (binding capacity), is similar in the various cellular membrane fractions. Since it is well known that the liver cell has far more intracellular membranes than plasma membranes, this observation suggests that there may be more binding sites for insulin in the cell interior than there are on the cell surface.

The biological significance of these intracellular binding sites for insulin is unknown. It has been postulated that certain of these intracellular binding sites, especially those on Golgi membranes, are precursors of those on plasma membranes⁵; this argument may also pertain to those sites on the rough endoplasmic reticulum. Conversely since it is unlikely that the insulin binding sites on the nuclear membrane and on the smooth endoplasmic reticulum are precursors of those on the plasma membrane, and since the present studies indicate that these intracellular binding sites for insulin are immunologically distinct from those on the plasma membrane, an alternative hypothesis can be made. We and others have either deduced or observed that both insulin and other polypeptide hormones may enter the intact cell^{15–20}. On the basis of these data, we have speculated that these intracellular binding sites for insulin may be involved in the regulation of several long term effects of insulin, such as RNA and DNA synthesis^{15,16}. Since the

Table 2 Effect of an anti-plasma membrane antiserum on ^{125}I -insulin binding to various cellular fractions

Fraction	^{125}I -insulin bound (% of total)		Inhibition of binding (%)
	No antiserum	Antiserum 1:200	
Plasma membranes (0.035)	1.14 ± 0.02	0.16 ± 0.006	86
Nuclei (1.03)	1.34 ± 0.15	1.36 ± 0.16	0
Nuclear membranes (0.12)	1.04 ± 0.01	0.86 ± 0.02	17
Smooth ER (0.23)	1.70 ± 0.08	1.50 ± 0.10	12
Rough ER (0.47)	1.91 ± 0.04	1.32 ± 0.04	31

For each fraction the protein concentration (shown in parentheses as mg ml^{-1}) was adjusted so that 1–2% of the ^{125}I -insulin was specifically bound. Incubation conditions were as described in Table 1 and Fig. 1. Binding is expressed as the mean $\pm$ standard deviation for triplicate determinations. Inhibition of insulin binding by the antiserum was significant in all fractions ($P < 0.05$) except nuclei.

relationship between the intracellular binding sites for insulin and the biological functions of insulin is unknown, further studies will be necessary to clarify the role, if any, of these intracellular binding sites.

We thank Drs Marvin D. Siperstein and Basil Rapoport for advice on this manuscript. These studies were supported by the Medical Research Service of the Veterans Administration, by a grant from Northern California Affiliate, Inc. of the American Diabetes Association, and by a grant (AM 19415 01A1) from the National Institutes of Health. R.V. was a recipient of a Fulbright International Fellowship.

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Received 31 May; accepted 22 August 1977.

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Inhibition of high-affinity choline transport in peripheral cholinergic endings by presynaptic snake venom neurotoxins

Use of purified snake venom neurotoxins with potent curari-mimetic action on peripheral cholinergic transmission (for example, α -bungarotoxin¹) in the study of the biochemical properties of the nicotinic cholinergic receptor has met with considerable success (see ref. 2 for review). Some of the crude venoms from which such toxins have been isolated also contain toxins which act presynaptically, such as β -bungarotoxin¹, notexin³ and taipoxin⁴. These three toxins inhibit the release of acetylcholine by a mechanism which is independent of the release process *per se*^{3–7}. We show here that in an *in vitro* membrane preparation⁸ derived from the cholinergic endings in *Torpedo* electric organ these and related toxins are potent inhibitors of the high-affinity choline transport system. These observations provide a biochemical explanation for the inhibitory action of these toxins on transmitter release as they effectively block re-synthesis of acetylcholine from choline.

Nerve terminal sacs (T sacs, originally termed synaptosomes⁸) were prepared from the electric tissue of *Torpedo marmorata* essentially as described previously⁸ (for a detailed account see

ref. 9). After a brief period of pre-homogenisation of this tough tissue in 0.8 M glycine using a Waring blender, homogenisation in a Potter–Elvehjem glass and Perspex homogeniser was possible. Uptake of ³H-choline into partially purified T sacs (fraction P₂) was measured using a Millipore filtration technique as described recently¹⁰. The effects of the various toxins and related proteins on choline uptake were tested in several ways. The concentration dependency of their inhibitory action was tested by simultaneous exposure of T sacs to ³H-choline (1 μ M) and toxins (varying concentrations) for 10–15 min before filtration. In other experiments T sacs were exposed to toxins before the addition of ³H-choline, and vice versa. In experiments designed to test the calcium dependence of the toxin action the 4.4 mM CaCl₂ of the normal *Torpedo* Ringer (for composition see ref. 10) was replaced by 4.4 mM SrCl₂.

Figure 1 shows that all three toxins (β -bungarotoxin, notexin, and taipoxin) that are known to have presynaptic effects are potent inhibitors of high-affinity choline transport. By contrast those with a postsynaptic action (α -bungarotoxin and *siamensis* 3)¹⁸ in the same concentration range were without effect (data not shown). Two other toxins (*Notechis* II-5 and *Enhydryna schistosa* myotoxin) which are structurally homologous to notexin and the taipoxin subunits^{7,11–13} are also very potent inhibitors. Experiments in which T sacs were exposed to taipoxin or β -bungarotoxin for 10–15 min at room temperature before the addition of ³H-choline show that this potentiates the inhibitory action of taipoxin 10-fold whereas that of β -bungarotoxin is not significantly altered. The increased potency of taipoxin is not due to increased binding of the toxin to the T-sac membrane but rather to a potentiating factor released from the tissue during incubation (M.J.D. and J.P.F., in preparation).

The shapes of the inhibition curves suggested that all of the inhibitory toxins except β -bungarotoxin were capable of producing full blockade. For notexin and *Notechis* II-5 it was not possible to demonstrate complete inhibition because of the increased nonspecific binding of ³H-choline to the Millipore filters at high toxin concentrations. In contrast to the retention of ³H-choline at lower toxin concentrations this binding was insensitive to the presence of hemicholinium-3 and thus probably represents a strong interaction between ³H-choline and filter-bound toxin. With β -bungarotoxin, inhibition was never more than 60% even at concentrations above 30 μ g ml⁻¹. In the presence of 100 and 200 μ g ml⁻¹ β -bungarotoxin nonspecific binding of ³H-choline to the filters was also observed and this obscured the real inhibition pattern of this toxin at these elevated concentrations. By extrapolation this effect was not sufficient to explain the partial blockade caused by β -bungarotoxin, however. The most potent uptake-blocker, notexin, had an IC₅₀ value (concentration for 50% inhibition) of about 4×10^{-11} M. In cases where comparison is possible (β -bungarotoxin, notexin and taipoxin) the range of concentrations in which inhibition was observed is similar to or lower than that previously reported for *in vitro* presynaptic inhibition based on physiological findings^{1,3,4,7}. The latency of the presynaptic toxins *in vivo* is well established. If the failure of transmission is due to termination of acetylcholine synthesis caused by inhibition of choline uptake one would not expect transmission to cease until the pre-existing pool of acetylcholine was exhausted. Comparisons of IC₅₀ values for choline transport in T sacs with the LD₅₀ values observed on intravenous administration to mice shows a high degree of correlation—particularly for the single-chain neurotoxins. The multiple-chain toxins, β -bungarotoxin and taipoxin, are more toxic to whole animals than might be predicted from their effects on choline transport.

All of the presynaptically-active neurotoxins shown in Fig. 1 are Ca²⁺-dependent phospholipases, and all of those which have been totally^{11,12} or partially^{7,13} sequenced are highly homologous to phospholipases A₂ from other snake venoms and from mammalian pancreas. We have therefore tested (Table 1) whether calcium is required for their inhibitory action on choline transport by performing some experiments in

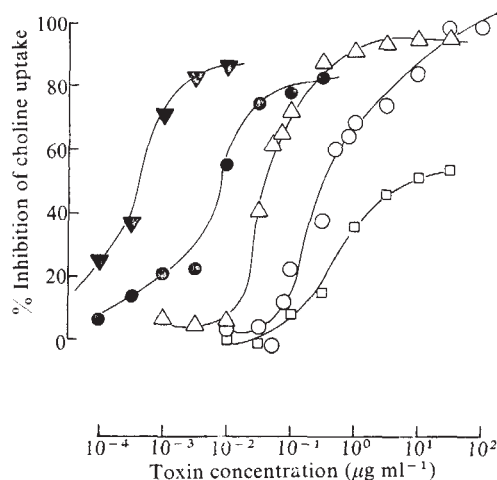


Fig. 1 Inhibitory action of presynaptic snake neurotoxins on high-affinity choline transport. ^3H -choline uptake into partially purified T sacs (fraction P_2) from *Torpedo* electric organ was measured for 10–15 min at room temperature with $1 \mu\text{M}$ choline exactly as described previously¹⁰. The effect of increasing concentration of the various toxins added at the onset of incubation with ^3H -choline is expressed as % inhibition of controls to which no toxin was added. Each point represents the mean of two or more independent experiments with duplicate or triplicate determinations in each case. Replication of individual points was good both within or between experiments. For any one point the standard deviation from the mean was $\leq 10\%$ for $n = (4-13)$ observations. The identities of the various toxins, assumed molecular weights (MW) and references to the details of their purification are as follows: $\blacktriangledown$, notexin¹¹, MW 13,500; $\bullet$, *Notechis* II-5¹², MW 13,500; $\triangle$, *Enhydrina schistosa* myotoxin¹³, MW 13,500; $\circ$, taipoxin⁷, MW 45,600; $\square$, β -bungarotoxin (a gift from Boehringer prepared as in ref. 17), MW 22,000. Toxin concentrations causing 50% inhibition in ng ml^{-1} were: notexin, 0.5 (3.7×10^{-11} M) (2 expts); *Notechis* II-5, 9 (6.7×10^{-10} M) (2 expts); *En. schistosa* myotoxin, 36 (2.7×10^{-9} M) (2 expts); taipoxin, 310 (6.8×10^{-9} M) (4 expts); β -bungarotoxin, 5,600 (2.5×10^{-7} M) or for 50% of maximum inhibition, 630 (2.8×10^{-8} M) (5 expts).

Ca^{2+} -free Sr^{2+} -*Torpedo* Ringer. Although substitution of Ca^{2+} by Sr^{2+} has little effect on choline transport in the absence of the toxins none of the toxins exhibited either inhibitory activity or phospholipase A activity in the Sr^{2+} medium, which suggests that the integrity of the catalytic site is required for the toxic action.

Experiments of the type shown in Fig. 2 indicate that these toxins do not cause gross lysis of the presynaptic nerve-terminal membrane in the conditions used. Furthermore, the influx of ^3H -choline is immediately and totally inhibited by the addition of taipoxin, but continued incubation does not lead to loss of ^3H -choline. In the same conditions, 80% of the

choline taken up remains as free choline in the cytosol¹⁴ and is readily released by agents which rupture the external membrane (see effect of crude bee venom phospholipase A in Fig. 2).

β -Bungarotoxin behaves like taipoxin except that the blockade is only about 50%, as mentioned above (Fig. 1). Another reason for rejecting gross lysis as an explanation for the inhibitory action of these toxins on choline transport is the very poor correlation between phospholipase activity and inhibitory potency. A more subtle mechanism of action involving factors other than or, at least, in addition to phospholipase A activity is therefore indicated.

Taipoxin is a ternary complex of three separate, but homologous peptide chains⁷ which differ strikingly in their charge properties. The α component is very basic, the β component is neutral, and the γ component is very acidic⁷. Previous studies at the neuromuscular junction have shown that the α component is the only one of the three which has appreciable blocking action by itself^{6,7}. The same applies for the effect of the individual chains on choline transport (Table 2). In fact, all of the presynaptic snake venom neurotoxins for which data are available are either very basic polypeptide chains or contain such a chain as an essential subunit. These observations indicate that a positive charge might be important for toxicity and that ionic binding to the external membrane, which presumably has a negative charge, might thus be a prerequisite for the action of these toxins. That a highly charged molecule like protamine can also block choline uptake (Table 2) supports this view, but whereas the inhibition by protamine is reversible the toxin-induced block is not (M.J.D. and J.P.F., in preparation).

We do not yet know how specific the action of these presynaptic toxins is. Recent observations on the inhibition of high-affinity choline uptake in rat brain synaptosomes by β -bungarotoxin¹⁵ agree well with our results and suggest that some part of the high-affinity uptake mechanism might constitute the 'target' for these toxins. Alternatively, the toxins might interact with sites elsewhere on the presynaptic membrane, but thereby cause some general perturbation which

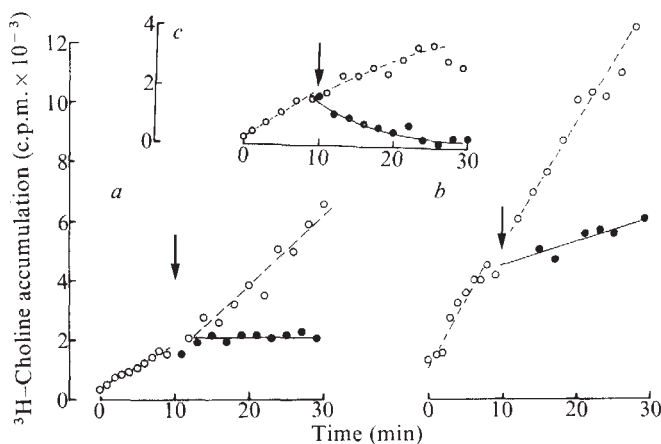


Fig. 2 Effect of toxin addition on ^3H -choline accumulated by T sacs. Fraction P_2 (about 2 mg protein in each case) from *Torpedo* electric organ was incubated in 32 ml of *Torpedo* Ringer containing ^3H -choline $1 \mu\text{Ci ml}^{-1}$ (specific activity 1 Ci mmol^{-1}) at room temperature. Samples of 1 ml were filtered at the times indicated and the accumulated ^3H determined. After incubation for 10 min the remaining T-sac suspension (22 ml) was transferred (two equal portions) to separate vessels containing either a small vol of Ringer (control) or the same vol of Ringer containing toxin. Samples (1 ml) were filtered alternately from each of these suspensions at 1-min intervals over the next 20 min; $\circ$, controls; $\bullet$, toxin treated. *a*, Taipoxin, $0.1 \mu\text{g ml}^{-1}$ final concentration. *b*, β -bungarotoxin, $1 \mu\text{g ml}^{-1}$. *c*, To demonstrate that gross lysis of T sacs does lead to loss of accumulated ^3H -choline, bee venom phospholipase A (Sigma) was added at a total concentration of $3 \mu\text{g ml}^{-1}$. Each plot represents the result with different T-sac preparations. Arrows represent times of toxin addition.

Table 1 Ca-dependence of toxin action on choline uptake

Toxin ($\mu\text{g ml}^{-1}$)	Choline uptake at $1 \mu\text{M}$ (% of controls)	
	Sr^{2+} -Ringer	Ca^{2+} -Ringer
Notexin (0.01)	91 ± 37	23 ± 6
<i>Notechis</i> II-5 (0.1)	111 ± 9	23 ± 3
<i>E. schistosa</i> myotoxin (0.2)	127 ± 3	33 ± 4
Taipoxin (3)	89 ± 15	9 ± 2
β -bungarotoxin (10)	111 ± 12	45 ± 6

Fraction P_2 was prepared from *Torpedo* electric organ and re-suspended in 'normal' *Torpedo*-Ringer (Ca^{2+} -Ringer) or one in which CaCl_2 was replaced by SrCl_2 with a final concentration of 4.4 mM (Sr^{2+} -Ringer). ^3H -choline uptake (10 min) was then measured ($20 \mu\text{g}$ protein per assay) in the presence and absence of toxins in both Ringer solutions as described in the legend to Fig. 1. Toxin concentrations were chosen so as to produce about 80% of their maximum inhibition (see Fig. 1). Results are expressed as % of the appropriate controls and are means $\pm$ s.d. of three individual determinations. The Sr-Ringer control was 93% of the Ca-Ringer control.

Table 2 Importance of positive charge for inhibition of choline uptake

Agent tested	Concentration range studied ($\mu\text{g ml}^{-1}$)	Concentration ($\mu\text{g ml}^{-1}$) causing 50% inhibition of choline uptake
α -chain Taipoxin (basic)	0.01–10	0.3
β -chain Taipoxin (neutral)	0.01–10	> 10 (90% of control at $10 \mu\text{g ml}^{-1}$)
γ -chain Taipoxin (acidic)	0.01–10	> 10 (120% of control at $10 \mu\text{g ml}^{-1}$)
Protamine (very basic)	0.1–100	0.4

Concentration-dependent inhibition of choline uptake into T sacs was measured as described in the legend to Fig. 1 using simultaneous exposure of T sacs to ^3H -choline and toxins (or protamine) for 10 min at room temperature. Protamine was used to test whether or not the binding of a positively charged macromolecule to the T-sac external membrane alone could cause inhibition of choline transport, which it does. Other experiments have shown that, unlike the toxins, the inhibitory influence of protamine is fully reversible. The molecular weights of the agents are as follows: α subunit, 13,800; β subunit, 13,500, γ subunit, 18,400 and protamine, $\approx 8,000$.

upsets membrane-associated processes including choline transport. That taipoxin and notexin can also interfere with vesicle recycling at the neuromuscular junction⁶ supports the idea of a generic attack on presynaptic membrane-dependent processes. It seems unlikely that blockade of choline uptake is a secondary effect of inhibition of vesicle recycling since for taipoxin the blockade is instantaneous (Fig. 2). A strong case for general membrane perturbation has been presented recently for β -bungarotoxin¹⁶. We are currently attempting to localise and identify the 'target' site by using radiolabelled toxins.

This work was supported in part by an EMBO Fellowship to J.P.F. We thank Dr E. Karlsson for *siamensis* 3 neurotoxin.

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High densities of benzodiazepine receptors in human cortical areas

THE presence of brain-specific benzodiazepine receptors in membranes from rat brain is now established^{1–4}. Highly significant correlations between the affinities of various benzodiazepines for the benzodiazepine receptor site in rat brain on the one hand and clinically predictive pharmacological activities in several species on the other, strongly suggest that the benzodiazepine receptor *in*

vitro is related to a physiologically relevant receptor for benzodiazepines *in vivo*. We report here that benzodiazepine receptors are also present in the human brain, that the cerebral and cerebellar cortical regions contain the highest densities of binding sites and that the receptors in human brain are very similar to those in rat brain.

The brains of four humans, two males and two females, aged 18, 32, 54, and 72 yr and dying from malformatio congenita cordis, ulcus duodeni, stenosis valvulae mitralis and arterio sclerosis were investigated. No patients had a recorded history of benzodiazepine treatment within 14 d of death. Autopsies were carried out 17–72 h after death and 0.4–1.8 g of brain tissue was excised and frozen at -70°C until analysis within 14 d (experiments with rats showed that the affinity constants and density of binding sites can be safely determined 1–3 d after cessation of lorazepam or diazepam treatment, and that brains, left for 6 h at room temperature and then for 3 d at 4°C , have normal receptors). White matter contamination of grey matter was kept to a minimum, and grey matter contamination of white was avoided. The regional distribution of specific ^3H -diazepam binding sites was similar in all the brains. Table 1 therefore presents the average values from two to four brains. The receptor binding assay² consisted of an osmotically shocked crude synaptosomal preparation which was incubated for 40 min at 0°C with 0.57 – 11.3 nM ^3H -diazepam (*N*-methyl- ^3H), $14.4 \text{ Ci mmol}^{-1}$ (kindly donated by Willy Haefely of F. Hoffman-La Roche). After incubation, the receptor–ligand complex was separated by filtration through glass fibre filters and measured by scintillation counting. All binding

Fig. 1 Correlation between K_i values for ^3H -diazepam displacement of 13 clinically active benzodiazepines (the average for frontal and cerebellar cortex, from Table 2) and clinically recommended dosages (Danish Physicians desk reference to pharmaceutical specialities¹⁶). The K_i -values for rat brain benzodiazepine receptors¹⁸ are also shown. The best curve fit was calculated by linear regression analysis. Numbers on graph indicate drug number in Table 2. *a*, $r=0.974$; $P<0.001$. *b*, $r=0.631$; $P<0.05$.

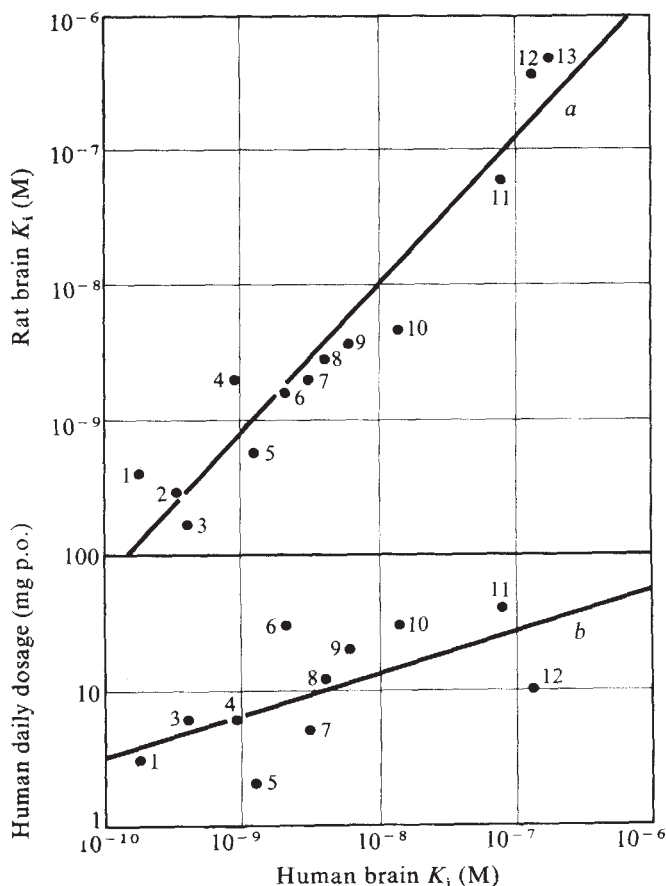


Table 1 Benzodiazepine receptors in various regions of the human brain

Brain region ²⁰	Specific ³ H-diazepam binding (c.p.m. per assay)	Density of ³ H-diazepam receptors (pmol per g protein)	K_D (nM)	IC ₅₀ (nM)	
				Diazepam	Clonazepam
Frontal lobe cortex (gyrus frontalis inf.)	960 ± 200 (3)	860 ± 130 (3)	3.5 ± 0.3 (3)	22 ± 8 (3)	6.6 ± 0.7 (3)
Frontal lobe cortex (gyrus centralis inf.)	730 ± 130 (4)	760 ± 90 (4)	6.8 ± 2.0 (4)	16 ± 4 (3)	5.6 ± 0.4 (3)
Occipital lobe cortex (gyrus occipitalis lat.)	1,280 (2)	840 ± 110 (3)	4.8 ± 0.8 (3)	11 (2)	5.9 (2)
Temporal lobe cortex (gyrus temporalis inf.)	870 ± 260 (3)	670 ± 150 (3)	4.9 ± 1.3 (3)	20 (2)	6.5 (2)
Cerebellar hem. cortex	580 ± 20 (3)	580 ± 40 (3)	4.2 ± 0.5 (4)	15 ± 3 (3)	5.2 ± 1.3 (2)
Vermis	350 (2)	760 (2)	5.7 (2)	24 (2)	3.7 (2)
Hippocampus	615 ± 190 (3)	670 ± 120 (3)	4.2 ± 0.6 (3)	14 (2)	5.2 (2)
Amygdala	165 (1)	510 (1)	9 (1)	13 (1)	2.3 (1)
Hypothalamus	250 ± 75 (3)	450 ± 120 (3)	7.4 ± 2.3 (4)	15 ± 4 (3)	8.4 ± 0.5 (3)
Thalamus	560 (2)	330 ± 55 (3)	4.6 (2)	20 (2)	4.0 (2)
Nucleus caudatus	450 ± 90 (3)	440 ± 90 (3)	4.3 ± 1.4 (3)	15 (2)	6.4 ± 0.9 (3)
Putamen + globus pallidus	455 ± 65 (3)	360 ± 60 (3)	4.1 ± 0.6 (4)	11 ± 0.3 (3)	5.2 ± 1.1 (3)
Nucleus dentatus	80 (2)	160 (2)	7 (2)	18 (2)	4.1 (1)
Corpus callosum	85 ± 20 (3)	110 (2)	20 ± 7 (4)	70 (2)	5.8 (2)
Pons	110 ± 5 (3)	160 ± 20 (3)	5 ± 3 (3)	40 ± 24 (3)	
Medulla oblongata	130 (2)	200 (2)	12 ± 6 (3)	30 ± 20 (3)	9.5 (2)
Medulla spinalis	90 (2)	210 (2)	7 ± 2 (3)	50 (2)	9.1 (2)

The value for 'specific binding' represents the control binding (c.p.m. per assay, corresponding to 10 mg original tissue) at 1.9 nM ³H-diazepam used for IC₅₀-value determinations (see Table 2). The receptor densities were determined by Scatchard analysis (0.57, 1.6, 4.5, 11.3 nM ³H-diazepam, each in triplicate). In all regions Ro 5-4864 (300 nM) displaced less than 50% of specific ³H-diazepam binding. Values are the mean ± s.e.m. of (*n*) different brains, each assayed once. Nonspecific binding of ³H-diazepam amounted to 9–60% (120–200 c.p.m. per 10 mg wet tissue, at 1.9 nM ³H-diazepam) of total binding, depending on the region.

values were calculated as specific binding, defined as binding displaceable by 3×10^{-6} M unlabelled diazepam.

Table 1 shows the regional distribution of specific benzodiazepine receptors in the human brain. The densities of binding sites (determined by Scatchard analysis) show marked regional variations. The frontal cortex, occipital cortex, cerebellar cortex, temporal cortex and hippocampus contained the highest densities of specific binding sites (~700–900 pmol per g protein); intermediate densities were noted in corpus striatum, globus pallidus and hypothalamus (inclusive mammillary body) while corpus callosum, nucleus dentatus and pons showed low densities. In independent assays, the amount of specific binding was determined using a fixed concentration of ³H-diazepam (1.9 nM). These values paralleled the receptor densities determined by Scatchard analysis. The high levels of specific ³H-diazepam binding in cortical areas and the low levels in pons and medulla are very similar to the distribution in the rat brain^{2,3}.

Neuropharmacological data suggest that the hippocampus is a significant site of benzodiazepine action although several other brain areas have also been implicated¹⁷. Our results suggest that in addition to hippocampus, cortical areas may be important for the action of benzodiazepines.

The apparent affinity constants (K_D) were about equal in most areas (3–7 nM) and similar to the K_D values for the rat brain receptor (2.6–3.6)^{2,4}. Only two areas, the corpus callosum and the medulla oblongata, exhibited K_D values appreciably above 7 nM indicating that the specific binding of ³H-diazepam in these areas, which also exhibited low binding, is different in nature compared with other brain areas. The Scatchard analysis of the different regions showed no tendency for binding to resolve into more than one component showing the presence of only one receptor type. A single receptor is also indicated by the similar 50% displacement values (IC₅₀) for clonazepam and diazepam in several brain regions, as well as parallel K_i values for 13 benzodiazepines in frontal cortex and cerebellar cortex (Table 2).

Brain-specific ³H-diazepam binding in the rat is characterised by high affinities for ³H-diazepam ($K_D \sim 3$ nM) and clonazepam, but low affinity for the pharmacologically inactive benzodiazepine Ro 5-4864 (ref. 2). In contrast, a specific ³H-diazepam binding site in homogenates of rat kidney and liver exhibits low affinity for ³H-diazepam ($K_D \sim 40$ nM) and clonazepam and very high affinity for Ro 5-4864 (ref. 2). The findings that clonazepam and diazepam have high, and Ro 5-4864 low affinities for the ³H-diazepam binding site in all human brain regions suggest that this site is analogous to the brain specific site in rat and different from the site in rat kidney and liver.

There were no clear correlations between the regional distribution of ³H-diazepam binding site and the regional distribution of dopamine⁵, dopamine receptors^{6,7}, noradrenaline⁵, noradrenaline receptors^{6,8}, dopamine- β -hydroxylase (in the rat)⁹, 5-hydroxytryptamine (5-HT)⁵, 5-HT receptors⁶, acetylcholine (in the cat)⁵, acetylcholine receptors⁶, choline acetylase^{5,6}, γ -aminobutyric acid (GABA)^{5,6}, GABA-receptors^{6,10}, glutamic acid decarboxylase^{6,11}, endorphins (unspecified¹² or rat¹³), opiate receptors^{6,14} and substance P (refs 15, 19). Only GABA, bicuculline binding sites (in the rat¹⁰), etorphine binding sites¹⁴ and the benzodiazepine receptors exhibit the rather unusual pattern of high levels in cerebral cortex and appreciable amounts in the cerebellar cortex.

The ability of several clinically active benzodiazepines to displace ³H-diazepam from the receptors (K_i values) from both frontal cortex and cerebellar cortex correlate with the recommended daily clinical doses (Fig. 1), with an *in vivo* test for human anti-anxiety¹⁷ ($r = 0.736$; $P < 0.025$) and with the K_i values for the rat brain receptor (Fig. 1). These results further demonstrate the similarity between the benzodiazepine receptors in human brain and those in the rat brain, and suggest that they are involved in the clinical effects of benzodiazepines.

Table 2 Inhibition of specific ³H-diazepam binding (1.9 nM) to human brain membranes by benzodiazepines

No.	Compound	K_i (nM)	
		Frontal cortex	Cerebellar cortex
1	Lorazepam	1.5	1.9
2	Flunitrazepam	3.5	3.2
3	Clonazepam	4.3	3.4
4	Diazepam	11	7.1
5	Estazolam	11	13
6	Flurazepam	21	21
7	Nitrazepam	36	25
8	Bromazepam	37	43
9	Tranxene	71	56
10	Oxazepam	150	120
11	Chlordiazepoxide	980	590
12	Medazepam	1,780	840
13	Ro 5-3636	1,910	1,410
14	Ro 5-4864	24,500	13,200

Serial dilution of benzodiazepines in duplicate were added to the high-affinity binding assays from either frontal cortex or cerebellar cortex. K_i values were calculated using the equation: $K_i = IC_{50}/(1 + (C/K_D))$; IC₅₀ is the concentration causing 50% inhibition of specific ³H-diazepam binding; C = ³H-diazepam concentration (1.9 nM) and K_D = affinity constant (3.5 and 4.2 nM, Table 1). Each value is the mean of two or three determinations with a range less than $\times 2.5$.

This work was supported by a grant from Statens Lægevidenskabelige Forskningsråd, Copenhagen. We thank A. M. Buhl and A. Stenberg Knudsen for technical assistance.

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Received 12 July; accepted 19 August 1977.

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Altered exploratory behaviour after 6-OHDA lesion to the dorsal noradrenergic bundle

IN spite of the considerable evidence about the behavioural functions of brain dopamine (DA), and especially the nigrostriatal tract¹⁻⁹, relatively little is known about the functions of noradrenergic (NA) systems. Although they were initially suggested to be involved in learning^{10,11} and the coding of reinforcement¹², this is now clearly no longer tenable¹³⁻¹⁷. Perhaps the best documented function of the dorsal noradrenergic bundle^{18,19} is in extinction situations¹³. The actual behavioural mechanism involved in these situations, and how it is altered by the lesion, remains complex although some progress has been made in its elucidation²⁰⁻²². We report here what may prove to be a new class of behavioural deficit produced by selective destruction of NA systems. It was found that the response to novelty was altered in two different test situations after the lesion.

Ten male albino Woodlyn rats weighing about 300 g, received 4 µg of 6-hydroxydopamine (6-OHDA) injected stereotactically into the fibres of the dorsal bundle. Ten control animals received sham operations. The animals were allowed to recover for two weeks before behavioural testing started. After testing the ten treated and four of the control animals were killed and their brains assayed for NA²³. This confirmed that the injection of 6-OHDA had produced severe depletion of forebrain NA (cortex-hippocampus to 3.9% of control values and hypothalamus to 25.5% of control). It is known from previous work using this same technique that no alteration occurs in the concentration of brain DA¹⁵. Behavioural testing consisted first of an examination of the exploratory behaviour in a complex, structured situation comprising a series of parallel runways²⁴, and second of the investigatory response of the animal to the introduction of a novel object into a familiar cage. Nine treated and nine control animals were deprived of food for 24 h, placed in the start box of the maze apparatus and a few seconds later the door leading to the alleyways was raised. Measures taken included the time to emerge from the start box into the alleyways, the number

of alleyways entered in successive 3-min periods for a total of 21 min and the total time spent in the goal box before consumption of the first of four food pellets present there in a small elevated food cup. The 21 min of alleyway exploration started as each animal emerged from the start box and so was not contaminated by any possible difference in the emergence time between the two groups.

The number of alleyways entered per 3-min period is shown in Fig. 1; treated animals showed considerably more exploration than controls. (Alleys entered in the total 21-min period; treated mean = 96.3, control mean = 65.6, Mann-Whitney²⁵ $U = 9$, $P < 0.01$.) The time course (Fig. 1) revealed that the difference lay not in the initial rate of exploration in the first 3 min in the apparatus, but in the habituation to the novel situation with continued exposure. The treated rats also took longer to emerge from the start box (treated mean = 104 s, control mean = 46 s, Mann-Whitney $U = 22$, $P < 0.05$) and took longer to consume the first food pellet following entry to the goal box (treated mean = 91.5 s, control mean = 20.6 s, Mann-Whitney $U = 15$, $P < 0.05$). Thus, it seems from this experiment that exploration may be increased by the 6-OHDA lesion, possibly by a failure to habituate to the novel environment; to test this further, a second experiment was carried out.

The same animals (ten treated and ten controls) that had been used previously were placed on an *ad libitum* diet for 2 d and then habituated to a wooden cage for 15 min. During this period the number of times the animals crossed the line dividing the cage in two along the long axis was recorded as an index of spontaneous locomotor activity. At the end of a 15-min period the animal was removed

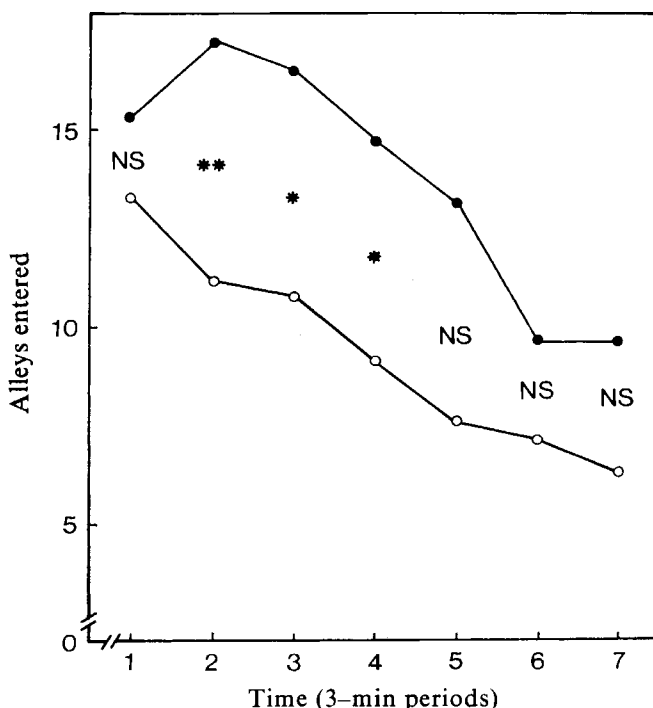


Fig. 1 The mean number of alleyways entered in a 3-min period plotted against successive periods for a total of 21 min in the 'parallel runway' apparatus for control and 6-OHDA-treated animals. Treated rats received stereotaxic injections of 4 µg of 6-OHDA base (obtained as 6-OHDA HBr.) dissolved in 2 µl of 0.9% saline with 0.3 mg ml⁻¹ ascorbic acid antioxidant infused at the rate of 0.2 µl min⁻¹ over 10 min at the coordinates AP 2.6, ML 1.1 and DV 3.7 according to König and Klippel. Controls were anaesthetised, positioned in the stereotaxic and burr holes drilled at these coordinates. The stars indicate that the two groups differed significantly at that time point. One star at the 5% level, and two stars at the 1% level. NS, No significant difference between the groups. ○, Control animals ($n = 9$); ●, treated animals ($n = 9$).

briefly and a novel object (cylinder 4.5 inch by 2.5 inch in diameter) was placed in one side of the apparatus and the rat replaced in the opposite side. The latency of the first approach to the novel object was noted, as was the total time spent in contact with the object over the next 15 min. Although no alteration in the latency to approach the novel object was found (treated mean = 7.4 s, control mean = 7.9 s), the time in contact was significantly higher for the treated animals (treated mean = 79.8 s, control mean = 40.0 s, Mann-Whitney $U = 20$, $P < 0.05$). This suggests that, in this simple situation as well, the lesion increased the amount of exploratory behaviour shown towards a novel object. The number of times that the rat initiated a bout of exploration aimed at the novel object was also recorded and this did not differ from controls (treated mean = 30.3, control mean = 22.2, $U = 39$, not significant) suggesting that the lesion had not altered the frequency of initiation of an exploratory bout, but since the total time in contact with the object was increased in the treated rats, had rather interfered with the ability to habituate to that object and stop exploration. The locomotor activity counts during the habituation phase before introduction of the novel object rule out either hyper- or hypoactivity as the cause of the increased exploration, since no difference was found between treated and control animals (treated mean = 29.7, control mean = 23.7, $U = 43$, not significant), and this has also been shown several times in other situations³⁰. A second locomotor activity measure taken on these same rats in photocell cages confirmed this (treated mean = 790 beam interruptions in 21 min, control = 976, t statistic = 1.61, not significant). We have since replicated these two exploration experiments with identical results in another group of animals depleted of brain NA.

The results reported here add to the few functions already demonstrated^{13,14,17} for NA systems. Since our lesions resulted in severe depletion of hypothalamic NA as well as cortical-hippocampal NA we cannot as yet say whether this effect is due to the dorsal NA projection from the locus coeruleus or the so-called ventral bundle arising in more posterior cell groups and innervating the hypothalamus^{18,19}. The behavioural mechanism involved must also remain indeterminate at this time. It is of interest, however, that animals with similar lesions have been shown to be resistant to extinction in a number of situations^{13,14,17}. Whether the elevated exploratory behaviour observed in the dorsal bundle lesioned animals similarly reflects impaired extinction of the exploratory response is not known. It is possible that the present observations may be yet another measure of the deficit which has been observed in other situations^{13,14,17}, or they may be due to damage to independent mechanisms possibly reflecting noradrenergic deafferentation in different terminal regions of the brain. Another possibility is that, as has been reported after locus coeruleus lesions^{26,27}, these animals may be less fearful and so tend to 'freeze' less and thus explore more. This seems less likely since the initial rate of exploration in the first 3 min in the maze apparatus did not differ from controls, suggesting that the immediate fear reaction was not altered. The subsequent habituation was retarded, however, suggesting perhaps that the treated animals might be unable to learn to overcome their fear. It has been suggested^{28,29} that the central emotional state produced by fear has similarities to that produced by frustration (caused by omission of an expected reward) and that the dorsal bundle lesioned rat may be impaired in learning about or remembering frustration (S. T. M. and S. D. Iversen, unpublished), although its general learning capacity in situations not involving frustration or similar states is unaltered¹³⁻¹⁷. An increase in the immediate baseline fear reaction is also counter-indicated inasmuch as no increase in the latency to approach the novel object immediately after its introduction was observed. Certain additional mechanisms may be tentatively

excluded since it has been shown that no deficit in internal inhibition (ref. 30 and S. T. M. and S. D. Iversen, unpublished), some forms of attention (S. T. M. and T. W. Robbins, unpublished), perseverance^{15,17,30} or general learning ability¹³⁻¹⁷ is present in these animals. The data reported here also rule out simple locomotor activity mechanisms. Further research will be required, however, to elucidate the basis of this alteration in exploratory behaviour found after depletion of forebrain NA.

S. T. M. is an MRC Fellow and the work was supported by the Medical Research Council. The technical assistance of Betty Richter is acknowledged. D. C. S. Roberts performed the 6-OHDA lesions.

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Received 27 June; accepted 15 August 1977.

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Cerebral lateralisation effects in visual half-field experiments

THE systematic study of behavioural differences related to differential functioning of the cerebral hemispheres has increased markedly in recent years. The studies of Sperry and his collaborators¹ on cerebral commissurotomy patients have stimulated many investigations into the hemisphere specialisations of normally functioning subjects. There are now many reports^{2,3} of statistically significant differences in hemisphere performance. Although hemisphere differences are consistent across independent experiments, they are small, occasionally inconsistent, and found within a limited experimental context, and so the generality of hemisphere differences remains to be established. We present here brief accounts of six experiments on cerebral lateralisation, four of which failed to find any differences and two which produced highly significant hemisphere differences. On the basis of this and published work, we believe that we can offer both a possible explanation for both the presence and absence of cerebral lateralisation effects in visual half-field experiments and also procedural guidelines that will produce cerebral lateralisation effects.

Much of the method for our six experiments is identical. Adult right-handed subjects fixated on a reference point in a three-field tachistoscope and indicated readiness to the experimenter. Stimuli were then shown for a period not exceeding 150 ms to the light visual field projecting to the left hemisphere (RVF-LH), or the left visual field projecting to the right hemisphere (LVF-RH), or with one member of a stimulus pair projected to each visual field (EVF). In all experiments, stimuli were located 2.5° of visual angle from the fixation point. Illumination level was 15 ft L.

Experiment 1: Stimulus materials were 96 words selected from a list of common Chinese terms using the norms developed by Liu and Chuang⁴ and their English equivalents (most English words used have A or AA Thorndike-Lodge ratings). The Chinese stimuli were all single characters which were drawn with a felt-tip pen and spanned 0.84° of visual angle. The English words (ranged from three to six letters) were done with Letraset 20 point Helvetica and spanned horizontally 0.84°–1.30° of visual angle at maximum. Forty-eight of the pairs were direct unambiguous translations and 48 were dissimilar terms. Presentation to RVF-LH, LVF-RH, and EVF were equal and randomised. Eight subjects fluent in reading Chinese and English responded by saying 'yes' if a stimulus pair had the same meaning and 'no' if they did not. Each of the 96 stimulus pairs was shown once. No term occurred more than once.

An analysis of variance of reaction times for making correct decisions revealed a statistically significant effect ($F(1, 7) = 19.90, P < 0.01$) for same-different judgments, indicating that decisions that two terms did not have the same meaning required more time than judgments of identity. Differences between visual fields and interactions did not differ appreciably from chance levels. Mean reaction times for experiments 1–4 are given in Table 1.

Experiment 2: At random, six of the stimulus pairs used in experiment 1 were discarded. The remaining 90 were then arranged as 30 English-English pairs, 30 Chinese-Chinese pairs, and 30 Chinese-English pairs (selected from the word pairs of experiment 1). Within each 30-pair set,

15 terms were identical and 15 different. Presentation to visual fields was as in experiment 1. Each stimulus pair was shown once and no terms were repeated. Subjects were 20 Chinese-English bilinguals, fluent at reading Chinese and English, and this time a manual (instead of oral) response was used.

Data analysis was identical to that in experiment 1. Only one comparison—that for judgments of same-different—reached an acceptable level of statistical significance ($F(1, 18) = 19.60, P < 0.001$). As was found in experiment 1, judgments of sameness take less time than judgments of difference. No visual field-hemisphere differences were present. The Chinese-English EVF data from experiments 1 and 2 were further partitioned into trials in which the Chinese was on the left and the English was on the right and vice versa. No reliable effect was obtained from this contrast either.

Experiment 3: Eight monolingual English subjects made judgments of same-different to the 30 English-English pairs and 30 Chinese-Chinese pairs used in experiment 2. Stimuli were shown once, with no repetition. Manual response reaction time was taken as dependent measure. Analysis revealed no statistically significant effects for any condition. No visual field-hemisphere differences were present and differences in response time to language (Chinese against English) and type of judgments (same against different) were not significant.

Experiment 4: Eight monolingual English subjects made judgments of same-different on stimulus pairs made up of only four Chinese characters and four English words. Within each language, every word was paired with every other word for 'different' stimulus pairs and with itself for 'same'. Presentation of either the Chinese-Chinese or English-English pairs was made only to RVF-LH and LVF-RH fields for 200 trials, which were distributed over five blocks with 40 trials per block. An analysis of variance of reaction times revealed a significant interaction effect of language by visual field by trial ($F(4, 28) = 4.00, P < 0.01$). Inspection of mean values indicated that reaction time by hemisphere effects increases systematically over trials and the data in the last block show a faster reaction time to English-English stimuli in the RVF-LH and a similar, though smaller, effect for Chinese-Chinese stimuli in the LVF-RH.

Experiment 5: Seventy-five subjects made judgments of same-different on pairs of figures selected from the spatial ability subtest of the Thurstone primary mental abilities test⁵. Stimulus pairs occupied about 2.5° of visual angle, were immediately adjacent, and at either 45° or 180° of rotational difference from each other. Stimuli were defined as 'same' when rotation in a clockwise or counter-clockwise direction allowed one stimulus to completely cover the other. When stimuli were mirror images, they were defined as 'different'. Seventy-two stimulus pairs were used, each pair shown once. The types of figures used each appeared twice, although no combination was ever repeated exactly. Reaction time was not measured in this experiment, the accuracy of response being the only parameter. Analysis of variance indicated no significant effects for visual field-hemisphere, type of judgment, or degree of rotation.

Experiment 6: Six pairs of stimuli were selected at random from the stimuli used in experiment 5. Three stimuli were 'same' and three were 'different', as defined in experiment 5. Eight subjects were shown the stimulus pairs, randomly presented to RVF-LH or LVF-RH only for 200 trials. An analysis of response accuracy showed that stimuli shown in the LVF-RH are judged more accurately than the same stimuli shown in the RVF-LH and that more errors were made in the 'same' pairs than in the 'different' pairs. Mean accuracy judgments for experiments 5 and 6 are given in Table 2.

Table 1 Mean reaction times for experiments 1–4

Condition			Experiments			
			1	2	3	4
			X	X	X	X
English-English	RVF-LH	Same	1,185	1,220	767	
		Different	1,310	1,017	806	
	RVF-LH	Same	1,176	1,264	715	
		Different	1,320	1,035	740	
	EVF	Same	1,084	1,048		
		Different	1,367	1,066		
Chinese-Chinese		Same	1,093	1,087	854	
		Different	1,141	1,084	796	
		Same	1,188	1,085	834	
		Different	1,098	1,127	794	
		Same	1,057	1,202		
		Different	1,132	1,107		
Chinese-English	RVF-LH	Same	1,341	1,175		
		Different	1,976	1,722		
	RVF-LH	Same	1,413	1,355		
		Different	1,955	1,606		
	EVF	Same	1,240	1,229		
		Different	2,099	1,584		

In experiment 1, response time was taken with an electronic voice key stopping an ms-timer activated when the stimulus was shown. In experiments 2–4, it was measured by pressing one of two buttons on a horizontal panel in front of the subject, so arranged as to allow comfortable arm placement. Subjects were instructed to press one button if the members of the pair shown were the same and the other button if they were different. The index finger of each hand was used to respond, with hand used for 'same' or 'different' responses counterbalanced across subjects. The timing mechanism was that used in experiment 1.

Table 2 Mean accuracy scores for experiments 5–6

LVF-RH	Same	6.43	29.23
	Different	7.24	36.49
RVF-LH	Same	6.06	18.12
	Different	7.73	33.12
EVF	Same	7.28	
	Different	9.15	

The accuracy scores were obtained by counting the number of correct responses made by subjects across all experimental trials. The highest score possible in experiment 5 is 12 and in experiment 6 is 50.

In summary, experiments 1, 2, 3 and 5 failed to detect any sign of hemisphere differences in judgment processes. These null effects should not be attributed to the insensitivity of the experimental design, since by merely changing the ratio of trials to experimental stimuli experiments 4 and 6 both found significant hemisphere function differences, similar to those reported by other investigators⁶.

In most interpretive models of cortical processing, an individual listening to the spoken word or engaged in reading, is hypothesised as having language information sent to the left hemisphere for processing, semantic analysis and understanding. An appropriate language response is hypothetically generated within the left hemisphere. Similar formulations are proposed for visuospatial processes within the right hemisphere. Our results cannot be explained within such interpretive models.

An activation model, such as that proposed by Kinsbourne⁷, also seems unable to account for our results. Given that all judgments will be semantic comparisons or pattern matches, the expectation from an activation model is that the hemisphere specialised for such processing will be activated and will show superior performance. No such process is evident in our experiments.

An alternative explanation is that lateralisation effects often reported in visual half-field experiments are not a function of immediate cognitive processing in specialised cortical areas, but do reflect hemisphere difference in memory storage location. The experiments reported here are consistent with such a formulation. If a subject has to evaluate new information on each trial, his reaction times or his response accuracy do not differ systematically with visual field presentation. Given such a reinterpretation of lateralisation phenomena, it is important to know if others have obtained similar results using the conditions described. A review of experiments reporting significant hemisphere specialisation effects indicates the number of stimuli used to range from 4 to 64, with the number of trials from 40 to 1,072. By contrast, published accounts which report no hemisphere specialisation differences^{8–10} have, in general, equal numbers of stimuli and trials. It would be of interest to reanalyse data from studies reporting lateralisation effects to see if the trends found in our experiments 4 and 6 are present there. Bryden¹¹ has reported such trends but to our knowledge they have not otherwise been investigated systematically.

The interpretation of these results seems straightforward: to obtain cerebral lateralisation effects, produce a condition that allows judgments to be made from memory. Experimentally, where a new evaluation and decision must be made on every trial, there is little evidence for any lateralisation of function. When the number of stimuli to be judged are so few that the subject may adopt a new strategy and carry out simple matching from a memory set of known size, then cerebral lateralisation effects are found.

These results suggest a number of interesting possibilities for the study of specific kinds of memory storage. Decreases in response time in relation to visual field presentations also suggest an interesting way of assessing when an item is firmly committed to memory. The most important

aspect of these findings, however, is the strong implication that (1) active cognitive processes are not lateralised, and (2) interpretative models which view active cognitive processing as being lateralised are not correct. The present work gives no support to the theory that, dealing with events which require active, immediate, continuous processing of a constantly changing flow of information is limited to one hemisphere at a time.

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Received 3 June; accepted 22 August 1977.

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Calcium-dependent regenerative responses in rods

NEURONES are thought to contain ion-selective channels whose opening and closing are regulated by the potential difference across the plasma membrane. It is thought that the sodium, potassium, and calcium currents which generate action potentials and modulate synaptic transmitter release pass through these channels. Investigations of voltage-dependent currents in various species have revealed remarkable uniformities in the ion selectivity and pharmacology of presumed membrane channels^{1–3}. The use of agents which block specific currents has provided a tool for the identification of voltage-dependent conductances in nerve membrane, which we have used to investigate vertebrate photoreceptors. We show here that when the retina of the toad *Bufo marinus* is superfused with Ringer containing 6–12 mM tetraethyl ammonium (TEA), rods generate oscillations and action potentials resembling the calcium spikes which have been described in various vertebrate and invertebrate preparations³. These experiments demonstrate that the rod membrane is not passive, as is often assumed, but contains at least one and probably two voltage-dependent conductances.

Retinas were removed from the eyes of dark-adapted toads under dim red illumination and were secured with receptors upwards in a perfusion chamber. Oxygenated Ringer flowed from a gravity-controlled perfusion system into a 0.03-ml pool above the retina at a rate of 0.5–1.0 ml min⁻¹. Silver-silver chloride electrodes were fixed in the chamber below the retina and above it in the perfusion pool to record the electroretinogram (ERG), and simultaneous recordings were made of intracellular responses with fine micropipettes with resistances of 200–400 MΩ. When the b-wave of the ERG reached a stable, dark-adapted threshold, a micropipette was lowered through the perfusion pool with an hydraulic microdrive. Just after reaching the retina, the pipette penetrated cells with response waveform, absolute

sensitivity, spectral sensitivity, and light adaptation characteristic of 'red' rods recorded in previous studies on toad eyecup⁴. Rods were stimulated with large-field, 516 nm light from a conventional, dual-beam photostimulator. Normal Ringer had the following composition: 96 or 106 mM NaCl, 1.8 mM Na₂SO₄, 0.13 mM NaHCO₃, 2.5 mM KCl, 1.2 mM MgCl₂, 1.8 mM CaCl₂, 5.6 mM glucose, and 3.0 mM HEPES, adjusted to pH 7.8 with approximately 2 mM NaOH (Ringer modified from ref. 5). All experiments were performed at approximately 22 °C.

Since TEA has been shown to block potassium currents in nerve membrane¹, we examined its effect on the rod photoresponse. We found that 6–12 mM TEA, either added to normal Ringer or substituted for NaCl, depolarises the rod membrane by 3–5 mV. In addition, the receptor potential returns more quickly to the baseline and is followed by 3–4 Hz, regenerative oscillations (Fig. 1*a*). The peak-to-peak amplitude of the oscillations is initially as large as 35 mV but decays to zero within 4–5 s. After extended perfusion in TEA-Ringer, the amplitudes of the oscillations decay to zero following the light flash more

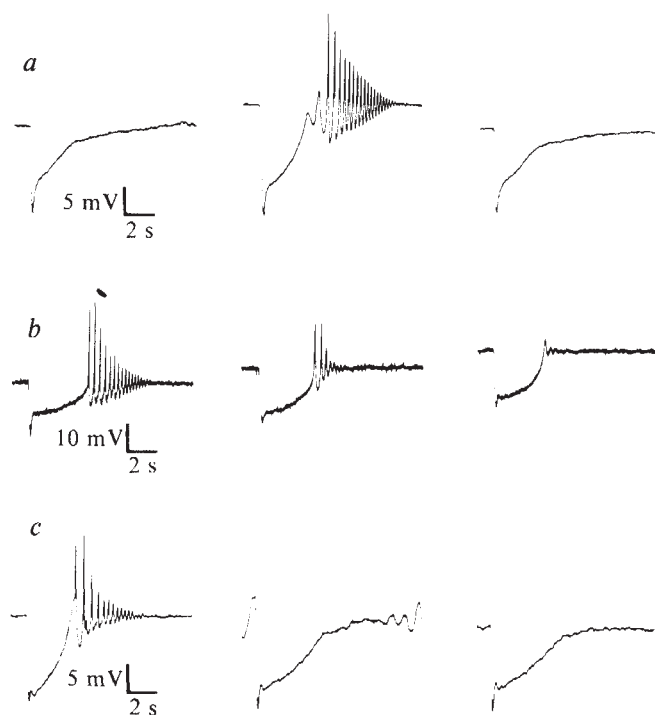


Fig. 1 TEA-induced oscillations in toad rods. *a*, *b*, and *c*, from different retinas. Each shows representative records taken from one continuous recording from a single rod in a dark-adapted retina. All responses are to 109-ms flashes of 516 nm, full-field illumination. *a*, Effect of TEA on rod responses. Left trace, response in normal Ringer (see text). Middle trace, after changing to Ringer containing 12 mM TEA chloride substituted for NaCl. Note 3.5 mV depolarisation of the dark membrane potential, faster decay of receptor potential toward baseline, and oscillations. Right trace, after return to normal Ringer. Membrane potential has repolarised, and the receptor potential waveform has returned to that observed initially. Stimulus intensity for each response was 9.3 log quanta per cm² per flash. *b*, Ca²⁺ dependence of rod oscillations. Left, response in Ringer containing 12 mM TEA and normal Ca²⁺ concentration (1.8 mM). Middle, in 12 mM TEA Ringer containing 1.0 mM Ca²⁺. Note 5 mV depolarisation of membrane potential and decreased amplitude and duration of oscillations. Right, in 12 mM TEA and 0.5 mM Ca²⁺. Membrane potential has depolarised 11 mV from that observed in TEA and normal Ca²⁺. Oscillations have nearly disappeared. Stimulus intensity: 9.8 log quanta per cm² per flash. *c*, Oscillations blocked by Co²⁺. Left, response in Ringer containing 12 mM TEA. Middle, in Ringer containing 12 mM TEA and 50 μM Co²⁺. Note slower decay of response to baseline and decreased amplitude of oscillations. Right, in Ringer containing 12 mM TEA and 100 μM Co²⁺. Oscillations have disappeared. Stimulus intensity: 9.8 log quanta per cm² per flash.

slowly than shown in Fig. 1, and eventually the oscillations become spontaneous in the dark. Similar oscillations are produced when the retina is superfused with Ringer containing 3 mM of the potassium channel blocking agent 4-aminopyridine⁶, or when the Ca²⁺ in the Ringer is replaced by Sr²⁺ or Ba²⁺. The oscillations can be recorded in the b-wave of the ERG, suggesting that the rods and second-order cells are all oscillating in phase. This is not unexpected as we stimulate the retina with a large, uniform field, and as the responses of the rods are tightly coupled by an extensive network of gap junctions⁷.

The effect of TEA on toad rods resembles that described for crustacean muscle fibres^{3,8,9} and for the presynaptic terminal of the squid giant synapse¹⁰. In these cells the membrane contains a voltage-dependent calcium conductance which, in the absence of TEA, is thought to be shunted by the development of a much larger, voltage-dependent potassium conductance. TEA seems to block a part of the potassium conductance, permitting the calcium current to become regenerative. Although we have no direct evidence that TEA is blocking a potassium conductance in rods, we have tested to see if a calcium current contributes to the oscillations in TEA Ringer by examining the effect of changes of external calcium concentration on the amplitude and duration of the oscillations. We found that as the calcium concentration is reduced, either by substituting Mg²⁺ for Ca²⁺ or by removing Ca²⁺ from the Ringer, the membrane potential depolarises, probably as the result of an increase in sodium conductance^{11,12}, and the oscillations become smaller and decay more quickly. This is shown in Fig. 1*b*. In 0.5 mM Ca²⁺ (right trace) the oscillations become quite small and, with prolonged perfusion, may vanish altogether. When all of the calcium is removed from the Ringer, the oscillations rapidly disappear. These effects are completely reversible if exposure to low calcium Ringer is not prolonged. In contrast, the oscillations are unaffected by the removal of Mg²⁺ from the 12 mM TEA Ringer.

Increases in [Ca²⁺] produce somewhat more complicated effects. We found that small increases in extracellular calcium cause small membrane hyperpolarisations, probably by decreasing the sodium conductance^{11,12}. Concomitant with this change in potential, there is a dramatic increase in the oscillation amplitude. In Ringer containing TEA and 2.7 mM (1.5 times normal) or 3.6 mM (twice normal) Ca²⁺, the oscillations of most cells become action potentials, up to 45 mV in amplitude and 60–100 ms in duration (Fig. 2). The action potentials are spontaneous in the dark but are interrupted by stimulation with light. The depolarising components of the action potentials are followed by 5 mV hyperpolarising undershoots, which decay to the baseline in 0.5–1 s. If the concentration of Ca²⁺ is increased to 5–16 mM, the rod is further hyperpolarised, and both the action potentials and oscillations are suppressed. We believe that the suppression of the spikes in high Ca²⁺ concentration may simply be the result of the hyperpolarisation of the membrane potential below the spike threshold. Our reason for thinking this is that large spontaneous action potentials can be recorded when high concentrations of Sr²⁺ are added to TEA Ringer containing normal Ca²⁺ (1.8 mM). For the rods, as for many other nerve and muscle cells³, Sr²⁺ seems to be able to substitute for Ca²⁺ as a current carrier in calcium action potentials. Unlike Ca²⁺, however, Sr²⁺ has little effect on the rod membrane potential. As the Sr²⁺ concentration is increased, the amplitude of the action potential increases continuously, and in 28 mM Sr²⁺ the spikes are nearly 60 mV.

To provide further evidence for the identification of the mechanism producing the oscillations, we studied the effects on the TEA-induced responses of agents known to block calcium channels^{2,3}. We found that the oscillations were reversibly blocked by the addition of low concentra-

tions of cobalt chloride (Fig. 1c). In $50\text{ }\mu\text{M}$ Co^{2+} (Fig. 1c, middle trace), the effect of TEA on the decay of the response is partially reversed (that is, the decay to the base line becomes much slower than in normal TEA Ringer), and the oscillations are smaller and begin several seconds after the decay of the photoresponse. In $100\text{ }\mu\text{M}$ Co^{2+} (Fig. 1c, right trace), the oscillations disappear. The oscillations are also reversibly blocked by Mg^{2+} , but the required concentration is much higher ($2.5\text{--}5\text{ mM}$). A greater sensitivity to Co^{2+} than to Mg^{2+} has been previously observed for calcium currents in crustacean muscle and for transmitter release^{3,13}. The drug D-600, which has been shown to block the late phase of calcium entry in squid axon¹⁴, also produces a reversible block of rod oscillations at a concentration of 10^{-4} M .

In contrast to Co^{2+} , Mg^{2+} and D-600, tetrodotoxin at $2\text{ }\mu\text{M}$, a concentration more than sufficient to block sodium currents in axons¹, has no effect on the rod oscillations. The substitution of choline chloride for NaCl in the Ringer eliminates the oscillations, but this procedure also produces a large hyperpolarisation of the rod membrane potential². In choline chloride Ringer containing 28 mM SrCl_2 , rods generate $35\text{--}40\text{ mV}$, spontaneous action potentials. This experiment shows that sodium is not necessary for the generation of regenerative responses in rods but does not exclude the possibility that sodium currents contribute to the oscillations in normal Ringer.

The results described above are not incompatible with the possibility that the oscillations are caused by an interaction between receptors and second-order cells, since changes in Ca^{2+} concentration or the addition of Co^{2+} or Mg^{2+} to the Ringer could also affect synaptic transmission. In fact, Normann and Pochobradský¹⁵ have reported spontaneous oscillations in the membrane potential of toad rods which they believe to be caused by such interactions. We have not been able to examine the possible relation between the spontaneous oscillations of Normann and

Pochobradský and the TEA-induced regenerative responses we report, as we have never observed spontaneous oscillations in rods superfused with normal Ringer. It is unlikely, however, that the regenerative responses we have described are caused by synaptic interactions. The addition to the Ringer of 2 mM sodium aspartate, which has been shown to block the responses of second-order horizontal and bipolar cells in the retina^{5,15-17}, has no effect on the amplitude or frequency of TEA-induced oscillations, even though in our experiments sodium aspartate blocks the simultaneously recorded b-wave of the ERG.

Our results suggest that the oscillations and action potentials produced by the rods in the presence of extracellular TEA are caused by regenerative calcium currents. We cannot as yet exclude a contribution to the oscillations from an inward-going sodium current; however, the dependence of oscillation amplitude on external calcium concentration, the extreme sensitivity of the oscillations to external Co^{2+} and D-600, and their insensitivity to tetrodotoxin all argue against a major role for sodium. Furthermore, the spikes in high Ca^{2+} TEA Ringer have a time course resembling that of calcium spikes in other systems³, being much slower in rise time and longer in duration than that of any sodium spike so far described.

Although we believe that calcium carries the inward current which produces the rising phase of the spikes and oscillations, we are uncertain of the mechanism for their falling phase. It seems unlikely that the repolarisation is caused by inactivation of the calcium conductance, as calcium inactivation in other systems seems to be too slow^{3,18,19}. We have tested for a possible role for a chloride conductance by replacing all the NaCl in the TEA Ringer with sodium isethionate, but we found no effect on the oscillations. We suggest that, in addition to a calcium conductance, the rod membrane contains a voltage-dependent or calcium-activated potassium conductance, which is only partially blocked by external TEA.

We have no evidence as to the location of the presumed calcium channels in the rod membrane or their possible functional significance, though we suppose that at least part of the calcium conductance is involved in synaptic transmitter release. Our recordings are probably made from the outer segment of the rod²⁰⁻²², but it is possible that the oscillations are generated near the synaptic terminal and spread passively to the distal end of the receptor.

We thank Mr Bernard Lacaille for constructing the perfusion chamber and photostimulator and Dr S. Hagiwara for reading a draft of the manuscript. This investigation began in Paris with the assistance of an exchange fellowship to G.L.F. from Harvard University and The Institut National de la Santé et de la Recherche Médicale, France. The work was supported by grants from the DGRST (74-7-0385) and CNRS (ATP 1288) to H.M.G.; from the Norton Simon Foundation, Fight For Sight, Inc. and the NIH (EY 01844) to G.L.F.; and by a postdoctoral traineeship (EY 7026) to F.N.Q.

Note added in proof: Measurements of the current-voltage curve of rods in normal Ringer show a region of outward-going rectification positive to the dark membrane potential which is markedly reduced in 12 mM TEA. Calcium-dependent regenerative responses have also been recorded from the presynaptic terminal of barnacle photoreceptors²³.

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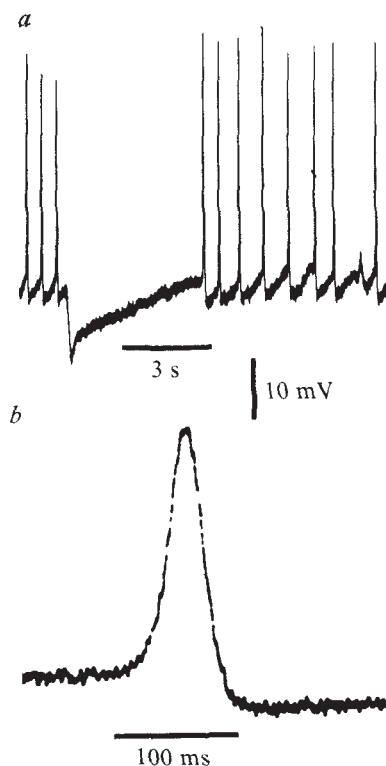


Fig. 2 *a*, Action potentials recorded from a toad rod during superfusion with Ringer containing 3.6 mM Ca^{2+} (twice normal concentration), no Mg^{2+} , and 12 mM TEA. Spikes are spontaneous in the dark but cease during the response to a 109-ms 516-nm , full-field flash of intensity $9.8\text{ log quanta per cm}^2\text{ per flash}$. *b*, Action potential from cell of Fig. 2*a* on an expanded timescale.

Received 31 May; accepted 8 August 1977.

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Ionic interactions in the potassium channel of squid giant axons

ONE of the simplest questions that can be asked about how ions cross biological membranes is whether or not they do so independently of one another. That is, is the probability of movement of an ion unaffected by the presence of other ions? In particular, with independent movement, the efflux of an ion should not be a function of the external concentration of that ion. Using long-lasting depolarisations, Hodgkin and Keynes demonstrated¹ non-independent movement of K^+ ions across the membrane of *Sepia* axons. Since the Na channel responsible for initiation of the action potential inactivates very rapidly on depolarisation, potassium ions were probably not moving through this pathway—even though the channel is permeable to them as well as to Na^+ ions. But since the K channels (partly responsible for termination of the action potential) also inactivate on prolonged depolarisation², the K efflux measured by Hodgkin and Keynes was presumably mainly through inactivated K channels and the so-called 'leakage pathway'. We report here on experiments that demonstrate non-independent ion movement through the activated K channel of the giant axon of *Loligo pealei*, using tetrodotoxin to block the Na channels, and short-step depolarisations to minimise K-channel inactivation.

Techniques for membrane voltage measurement and control have been described previously³. The axons were perfused internally with a solution containing 400 mM potassium labelled with ^{42}K . The artificial seawater (ASW) bathing the axons contained various K concentrations up to 75 mM. All experiments were done with a holding potential of -78 mV, and the data obtained with 20–40 mV depolarisations lasting for 20–30 ms. These pulses opened a sufficient number of K channels to produce measureable flux rates, yet were small enough to minimise K loading of the periaxonal space. Even so, some K loading into the 5 or 20 mM K-ASW solutions was detected from the reversal potential at the end of the depolarising pulse. To obtain a 'mean' value of external K during the pulse, we averaged the nominal K concentration in the ASW and the value computed from the reversal potential. Very little loading was observed for the 'high K' solutions. More refined averaging procedures would not alter the conclusions of the study.

Figure 1 shows ^{42}K efflux into ASW containing either 20 or 5 mM K. As expected, resting ^{42}K efflux from this hyperpolarised axon ($2-4$ pmol $cm^{-2} s^{-1}$) was lower than that reported for axons at rest^{4,5}. For a period of 7.5 min the membrane voltage was clamped once every 300 ms to a potential of -53 mV for 30 ms. The isotope efflux quickly increased during this period to a constant value, and rapidly returned to the low values following cessation of the pulses. With the membrane potential still maintained at -78 mV, the external solution was then changed to one containing only 5 mM K. After a 7.5-min control period, the same stimulation procedure was repeated. The efflux during repetitive

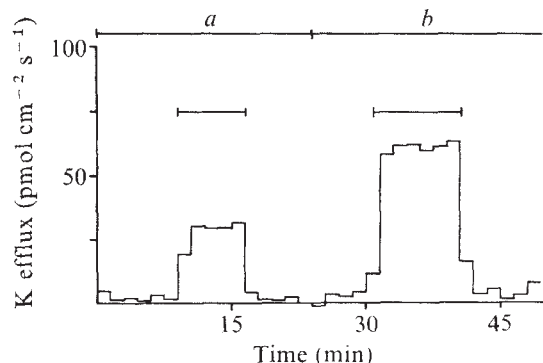


Fig. 1 Potassium efflux from an axon perfused internally with a solution containing 400 mM K^+ (labelled with ^{42}K) into artificial seawater (ASW) containing 20 (a) or 5 (b) mM K^+ . The perfusion solution contained (in mM): K glutamate, 320; K F, 50; sucrose 260; K phosphate, 30; pH 7.3. Perfusion pressure was produced by a motor-driven syringe delivering about $2 \mu l \min^{-1}$. ASW composition was as follows (in mM): NaCl + KCl, 430; $MgCl_2$, 25; $MgSO_4$, 25; $CaCl_2$, 10; EDTA, 0.2; Tris-HEPES (pH 7.8), 5. ASW was delivered, at 2.7 ml min^{-1} , to the centre of the experimental chamber (volume ~ 0.5 ml) by a peristaltic pump, removed by two pumps (0.9 ml min^{-1} each) through outlets located 3 mm on either side of the inlet, and collected in 1.5-min aliquots. The remainder of the flow was removed by suction through outlets located at the outermost edges of the chamber, and discarded; this was done in order to prevent collection of ^{42}K originating from imperfectly clamped regions of the axon ('end-effects'). The axon membrane voltage was held at -78 mV throughout the experiment, except during the periods indicated by the horizontal bars, when the axon was depolarised by a 25-mV, 30-ms rectangular pulse every 300 ms.

depolarisation was about twice that in the 20-mM solution; any spontaneous deterioration of the axon could only have caused an underestimation of this difference. Our voltage-clamp records showed little or no effect of external potassium on the kinetics of opening or closing of the K channels.

Figure 2 shows a similar record for another fibre, first in 50 mM K and then in 20 mM K-ASW, depolarised once every 200 ms by 40 mV for 30 ms. Since many more K channels are opened at -38 mV than at -53 mV, extra efflux here was much larger than that in the experiment of Fig. 1. Again, the efflux was greater into the solution containing the lower K concentration. After about 45 min, 2 mM 4-aminopyridine was added to the ASW. This compound is known to block the 'late' (that is, potassium) channels of giant axons^{6,7}. In the present experiment, the late outward current was reduced to less than 10% of control; extra ^{42}K efflux was also reduced to very low levels by this treatment, suggesting that a very large proportion of the measured flux in our experiments took place through K channels rather than 'leakage' pathways.

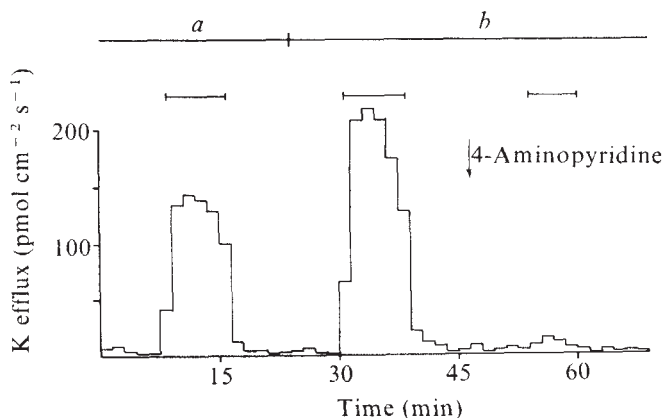


Fig. 2 Effect of external potassium ions and 4-aminopyridine on ^{42}K efflux. Experimental procedures were as in Fig. 1. Holding potential was -78 mV throughout the experiment. During the periods indicated, the axon was depolarised by a 40-mV, 30-ms rectangular pulse every 200 ms. a, 50 mM K^+ ; b, 20 mM K^+ .

The results of our experiments are summarised in Fig. 3. The ^{42}K efflux (normalised to the value for 20 mM K-ASW) is plotted against external K concentration for two values of the depolarising pulse (30 and 40 mV). In both cases ^{42}K efflux decreases with increasing external $[\text{K}]$, in violation of the independence principle. The relative inhibitory effect of external K on ^{42}K efflux, however, seems stronger for smaller depolarising pulses. (In additional experiments with 20-mV pulses, this trend was continued.)

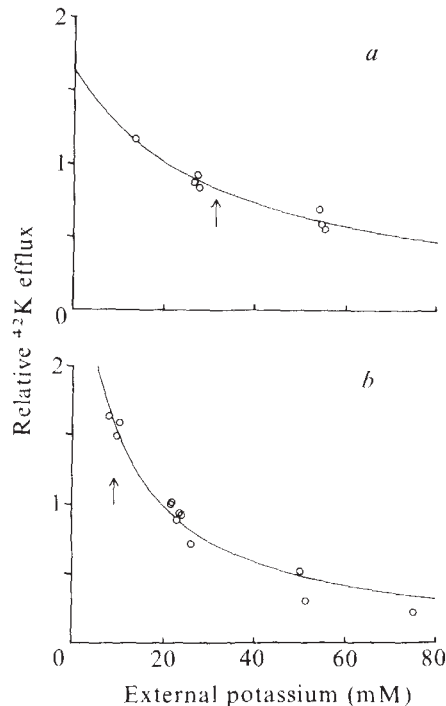


Fig. 3 Relative inhibition, by potassium in the seawater, of ^{42}K efflux through K channels of squid giant axon, for two different clamping pulse sizes. (See Fig. 1 for experimental procedures.) For each of the two pulse sizes: 40 mV (a), 30 mV (b), the fluxes were scaled to unity at 20 mM external K. Both sets of data were adequately fitted by simple adsorption isotherms of the form

$$\text{Flux} = \text{Flux}_{\text{max}} K_i / (K_i + [\text{K}]_o)$$

where K_i is the dissociation constant of a monomolecular reaction between external potassium and some site in the K channel, which results in blockage of ^{42}K efflux. The respective K_i values are indicated by arrows on the graphs. The apparent affinity of the channel site for external potassium is higher at the more negative clamping potential. This trend was continued in a few experiments (not shown) with 20-mV pulses (at -58 mV clamping potential).

The nature of the interaction of external K^+ ions with the K channel of squid axon membrane is unknown. Our data are consistent with the single-file model described by Hodgkin and Keynes for K^+ flux across steadily depolarised *Sepia* nerve membrane, but more work is needed to test detailed models. The most economical description of our data is to say that the activated K channel contains a site which, when occupied by external K, prevents efflux of internal ^{42}K . The dissociation constant of the potassium-site complex is of the order of 10^{-2} M, and decreases with increasing membrane potential.

This work was supported by the US NIH.

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Received 20 June; accepted 8 August 1977.

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Permeant cations alter endplate channel characteristics

At amphibian neuromuscular junctions, the binding of acetylcholine to postsynaptic receptors opens endplate channels. An endplate current (e.p.c.) is generated as sodium and potassium ions flow through these channels according to their electrochemical gradients¹. In normal conditions the e.p.c. is a net inward current of predominantly sodium ions. It has been shown that the lifetime of endplate channels is affected by temperature^{2,3} and membrane potential³, depends on the nature of the cholinergic agonist^{4,5} and can be influenced by various chemicals and drugs^{6,7}. But, it is not known what normally determines the lifetime and conductance of an open channel. We report here that the nature of the permeating cations that generate the e.p.c. affects the lifetime and conductance of endplate channels. These observations indicate that these cations influence the mechanisms that determine channel behaviour.

Endplate channel characteristics were compared for three different permeating cations—sodium, lithium and caesium. Normal extracellular solution (sodium solution) contained 115 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl_2 and 3 mM phosphate buffer. Lithium and caesium solutions were made by replacement of NaCl with 115 mM LiCl or 115 mM CsCl respectively. The pH of all solutions was 7.2 and experiments were performed at 20 °C.

Spontaneous miniature end-plate currents (m.e.p.cs) and current fluctuations (noise) generated by iontophoresis of acetylcholine were recorded in voltage-clamped fibres in sartorius muscles of toads (*Bufo marinus*) using techniques described previously^{8,9}. In most cases muscles were glycerol-treated to prevent contraction when fibres were depolarised. For analysis of acetylcholine noise, 8,192 or 16,384 data points, filtered by two low-pass, cascaded Butterworth filters in series (40 dB per decade roll-off) with cut-off at 800 Hz were sampled at 2 kHz. A fast Fourier transform method was used to analyse the frequency components of the noise and determination of single channel conductance and average lifetime was based on the model and theory described by Anderson and Stevens³. Lorentzian curves were fitted to data using a least squares fitting method.

In the solution containing lithium (lithium solution), the time constant of decay m.e.p.cs was significantly greater than the normal extracellular solution. This is illustrated in Fig. 1. Averages of 20 m.e.p.cs in normal (sodium) solution and 20 m.e.p.cs in lithium solution are shown in Fig. 1a. These m.e.p.cs were recorded in a fibre voltage-clamped at a membrane potential (V_m) of -70 mV. The decay phase remained exponential in lithium solution as is clear from the semilog plots of the decay phases of the same currents shown below (Fig 1b). In this experiment the average decay time constant (τ_d) was 2.5 ms in sodium solution and 3.3 ms in lithium solution. The lengthening of m.e.p.cs seen in lithium solution could be reversed by returning to sodium solution. In addition to the increase

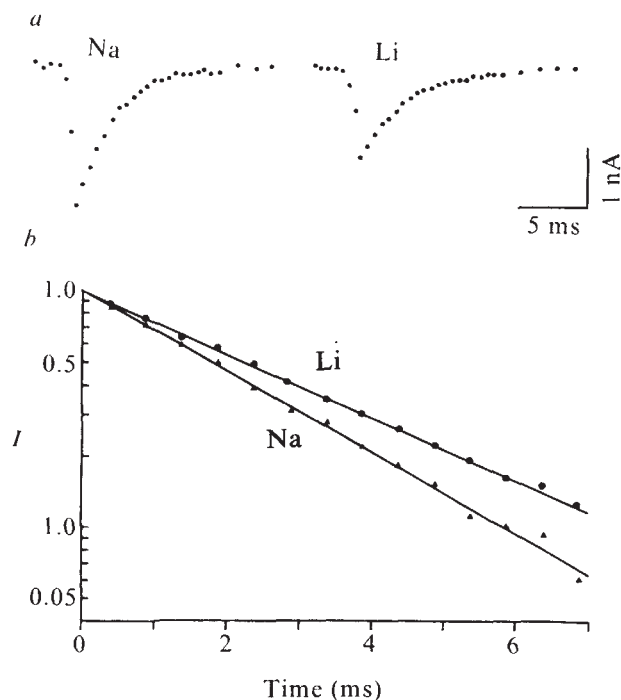


Fig. 1 Miniature endplate currents recorded in a muscle fibre voltage-clamped at -70 mV in sodium (Na) and lithium (Li) solutions. *a*, Averages of 20 m.e.p.s in the two solutions. *b*, Semilogarithmic plots of the decay of the averaged m.e.p.s (note normalised peak amplitudes) in sodium ($\blacktriangle$) and lithium ($\bullet$) solutions. The fitted lines are regression lines (correlation coefficients 0.999 and 0.998) and gave time constants of 2.5 ms and 3.3 ms in the sodium and lithium solutions respectively. (Temperature 20°C .)

in decay time constant, there was a decrease in the average amplitude of m.e.p.s in this experiment from 2.3 ± 0.09 nA in the sodium solution to 1.46 ± 0.06 nA in lithium solution (mean $\pm$ s.e.m.). This depression of m.e.p.c. amplitude was also reversible. The reduction in m.e.p.c. amplitude could not be explained by changes in acetylcholine null (reversal) potential (ϵ_s). ϵ_s was directly measured in each of the solutions by determining the potential at which there was no increase in current when acetylcholine was applied iontophoretically to an endplate. In seven fibres in lithium solution, the null potential was -6.1 ± 1.1 mV and in 15 fibres in sodium solution the null potential was -2.9 ± 0.5 mV. The increase in the time constant of decay and decrease in amplitude of m.e.p.s caused by lithium were consistent with the increased lifetime and decreased conductance of individual endplate channels as determined by analysis of acetylcholine current noise. Logarithmic plots of spectral density against frequency obtained at the same endplate are shown in Fig. 2 for sodium (Na) and after exposure to lithium solution (Li). In this fibre, voltage-clamped at -70 mV, the average lifetime of endplate channels (τ_n), calculated from $\tau_n = 1/2\pi f_c$ (f_c = half power frequency) was 1.5 ms in sodium solution and 2.5 ms in lithium solution. The current flow through individual channels (γ_i), determined both from the current spectral density at low frequencies and from the variance of current noise, was found to be reduced in lithium solution. Using the measured null potential, γ_e was calculated from $\gamma_e = \gamma_i / (V_m - \epsilon_s)$. Results obtained in several experiments are shown in Table I. It can be seen that lithium caused a significant reduction in γ_e from 26.7 pS to 17.7 pS and this probably explains the reduction in m.e.p.c. amplitude caused by lithium (Fig. 1).

In experiments in which sodium chloride was replaced with caesium chloride in the extracellular solution, m.e.p.s were increased in amplitude and their time constant of

decay was reduced. The acetylcholine null potential in caesium solution was 0.1 ± 0.9 mV (measured in five fibres). From analysis of acetylcholine noise it could be shown that the increase in amplitude and reduction in the time constant of m.e.p.s in caesium were associated with changes in endplate channel characteristics. In one fibre exposed first to sodium, then to caesium solution the average lifetime of endplate channels was reduced from 1.21 ms to 0.89 ms while endplate channel conductance increased from 28.2 to 35.0 pS (clamp potential -50 mV). In four fibres voltage-clamped at -50 mV in caesium solution, average endplate channel lifetime was 0.98 ± 0.05 ms and endplate channel conductance was 34.7 ± 2.1 pS from power spectra and 33.3 ± 2.6 pS from noise variance.

The results indicate that these cations which must be passing through open endplate channels to generate the endplate current, change the conductance and lifetime of the channels. The sequence of average channel conductances was $\text{Li} < \text{Na} < \text{Cs}$ and the conductance ratios were 0.7 : 1 : 1.3. Null potentials also became more positive in accord with this sequence. It is interesting and perhaps significant that the sequence of mobilities of the ions in free solution is also $\text{Li} < \text{Na} < \text{Cs}$ with free solution mobility ratios¹⁰ of 0.77 : 1 : 1.56 at 20°C . If the conductance of an endplate channel is determined by free-solution mobility of ions flowing through it, the channel may well be a relatively large, water filled pore. Of course, the similarity between the channel conductance, null potentials and mobility sequences may be only a coincidence. It could be that ions alter endplate channel conductance by directly affecting the structure that forms the channel and this explanation might apply also for the effects of the ions on endplate channel lifetime. The sequence of channel lifetimes was $\text{Li} > \text{Na} > \text{Cs}$ and the lifetime ratios were 1.4 : 1.0 : 0.7. Interestingly, there is a reciprocal relationship between channel lifetime and conductance (at least with the three ions tested). This may also be coincidental and the changes in channel lifetime may reflect separate and independent effects of these ions on the mechanism that determines channel lifetime. Whatever the mechanism and

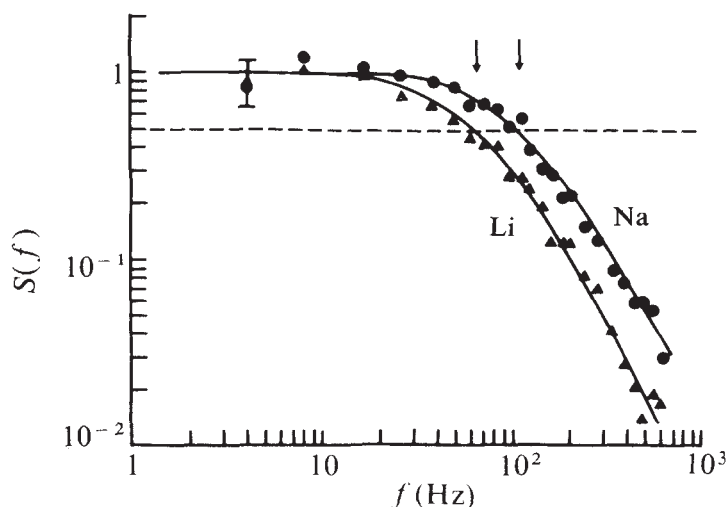


Fig. 2 Plots of normalised current spectral density $S(f)$ against frequency f (Hz) obtained from a fibre voltage-clamped at -70 mV in sodium solution ($\bullet$) and after 30 min in lithium solution ($\blacktriangle$). $S(f)$ was calculated at 4-Hz intervals from the ensemble average of spectra from 16 segments of noise containing 512 points sampled at 2 kHz (cut-off filter 800 Hz, 3dB down). The downward and upward error bars show 1 s.e.m. at 4 Hz for the Na and Li curves respectively. Points at frequencies above 8 Hz are a segment average of adjacent values of $S(f)$. The solid lines were obtained by least squares fits of the points to a single Lorentzian and the arrows denote the half-power frequencies (63 Hz in Li solution, 107 Hz in Na solution). Spectral densities were normalised for comparison. Asymptotic spectral densities were 0.18×10^{-21} A²s (lithium) and 0.32×10^{-21} A²s (sodium).

Table 1 The average lifetime (τ_N) and conductance (γ_g) of endplate channels in muscle fibres voltage-clamped at -50 mV in solutions containing 115 mM LiCl, NaCl or CsCl

Solution	Li	Na	Cs
n	7	7	4
τ_N (ms)	1.91 ± 0.07	1.38 ± 0.11	0.98 ± 0.05
γ_g (pS)	17.7 ± 1.8 (16.7 ± 1.5)	26.7 ± 0.80 (26.2 ± 1.1)	34.7 ± 2.1 (33.3 ± 2.6)

Results were obtained from power density spectra except for the values of γ_g in brackets which were calculated from noise variance. An average ± 1 s.e.m. was obtained from n experiments. (Temperature 20°C .)

sites of action of these permeant cations, it is clear that they have significant effects on endplate channels. An understanding of the cause of these effects should provide further information about processes that determine endplate channel lifetime and conductance.

This work was supported by a grant from the National Health and MRC of Australia. D.V.H. was supported by a grant from the Clive and Vera Ramaciotti Foundation. We thank Dr P. H. Barry, K. Takeda and D. J. Adams for useful discussions.

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Received 10 June; accepted 12 August 1977.

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Evidence for the Clara cell as a site of cytochrome P450-dependent mixed-function oxidase activity in lung

THE cellular localisation of pulmonary mixed-function oxidase (MFO) activity has aroused considerable interest, especially because of the increasing incidence of lung disease of environmental origin—including cancers—and the realisation that many chemical toxins and carcinogens require MFO-catalysed activation. The precise identification of pulmonary cellular populations possessing MFO activity has previously met with little success, however, probably because of the complexity and heterogeneity of the lung, which has been estimated to contain over 40 different specific cell types¹. In this communication I describe studies with the pulmonary toxin, 4-ipomeanol (1-[3-furyl]-4-hydroxypentanone)², which indicate that a highly reactive metabolite of this naturally occurring furan derivative is preferentially formed and covalently bound in pulmonary non-ciliated bronchiolar (Clara)³ cells, leading to their necrosis. Since previous studies⁴ have established that the toxic metabolite formed *in vivo* during cytochrome P450-dependent oxidative metabolism of 4-ipomeanol is formed *in situ* in the lung, and does not reach the lung by way of the circulation, the present results indicate that the Clara cell is a primary locus of P450-dependent MFO enzymes in lung.

Covalently bound 4-ipomeanol metabolite was visualised in the lung using an autoradiographic technique and Fig. 1 shows examples of the striking appearance of the autoradiograms. As indicated by the accumulations of black photographic granules,

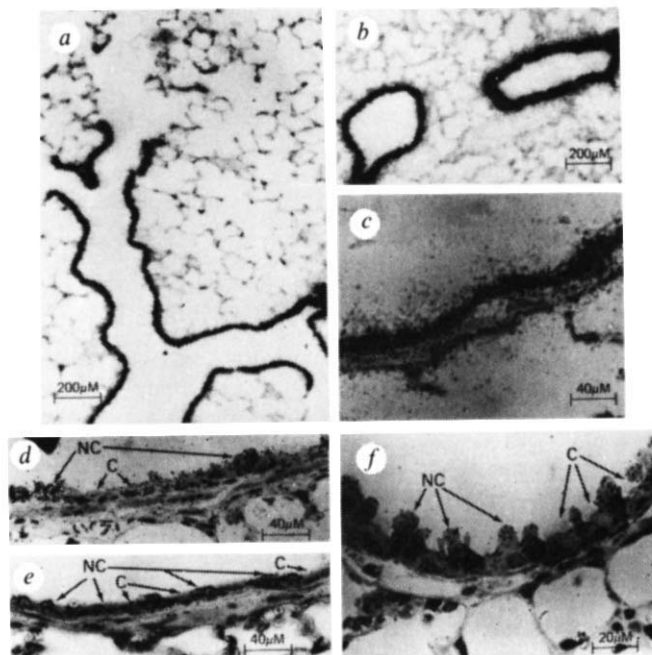


Fig. 1 Representative autoradiograms from lungs of rats (Sprague-Dawley, male, 100–120 g), mice (NIH-GP, male, 20–25 g), and hamsters (Golden Syrian, male, 60–70 g) given toxic doses of radiolabelled 4-ipomeanol (20 mg kg^{-1} rat or 35 mg kg^{-1} mouse and hamster) intraperitoneally. Generally-labelled ^3H -4-ipomeanol (60 mCi mmol^{-1}) was prepared by catalytic ($\text{Rh}/\text{Al}_2\text{O}_3$) tritiation with tritiated water. 5 - ^{14}C -ipomeanol (2 mCi mmol^{-1}) was prepared using 1 - ^{14}C -propylene oxide². The animals were killed using pentobarbital 2 or 16 h after receiving the toxin and the lungs fixed *in situ* by intratracheal perfusion with 3% glutaraldehyde buffered with 0.1 M sodium cacodylate. Lungs were removed, cut, washed and dehydrated using a standard ethanol gradient, followed by a final wash with propylene oxide. Radioactivity remaining in the tissue after this procedure represented only the covalently bound 4-ipomeanol metabolite and could not be removed by further washing with aqueous or organic solvents or by organic solvent extraction of samples solubilised in 1 N sodium hydroxide. Samples of the specimens were embedded in paraffin or Epon 812 resin. Sections of 4–5- μm thickness were cut from the paraffin blocks and mounted on glass slides, and 2- μm thick sections were cut from the Epon blocks and mounted similarly. The slides were dipped in fresh Kodak NTB-2 photographic emulsion and placed in the dark for 2 or 4 weeks for tritium or ^{14}C -labelled samples, respectively. The exposed slides were developed and stained with toluidine blue. Frames *a*, *b*, *c* and *d* are rat lungs, frame *e* is hamster lung, and frame *f* is mouse lung. NC (arrows) identify non-ciliated bronchiolar (Clara) cells, and C (arrows) identify ciliated bronchiolar cells. The lung specimens were taken from animals 2 h after dosing with the toxin. Specimens shown in *a*, *b*, *c* and *e* were embedded in paraffin, the others were embedded in Epon resin. Scale bars: *a*, *b*, 200 μm ; *c*, *d*, *e*, 40 μm ; *f*, 20 μm .

the covalently-bound 4-ipomeanol metabolite was heavily concentrated in the lining cells of the airways. Similar results were obtained with both tritium and ^{14}C -labelled 4-ipomeanol, indicating that the binding did indeed represent covalently-bound metabolite, as previous biochemical studies have indicated, and was not due to nonspecific tritium exchange or carbon incorporation. Comparison of frames *a*, *b* and *c* from animals receiving the ^{14}C -labelled toxin, with *d*, *e*, and *f* from animals receiving the tritiated toxin, however, illustrates that greater resolution can be obtained using the tritium derivative, especially combined with Epon embedding and thinner sectioning. For example, in *c*, where the ^{14}C derivative was used, there seems to be some specific, but not precisely definable, cellular labelling; in *d*, *e* and *f*, however, it is possible to distinguish between the several types of bronchiolar lining cells³ and to appreciate the cellular specificity of the labelling.

In all three animal species tested, the Clara cells³ of the small airways were specifically alkylated by 4-ipomeanol (Fig. 1 *d*, *e*, *f*). In addition, in other specimens taken from animals 16 h after dosing with the toxin, a striking necrosis of the Clara cells was seen, whereas the adjacent ciliated bronchiolar cells, and the other major

pulmonary parenchymal cells were not necrotic (Fig. 2). Electron microscopic studies have confirmed these findings. It thus seems that the Clara cell is a specific cellular target of 4-ipomeanol. It is likely that pathological features of 4-ipomeanol poisoning which have been described previously in cattle⁵ and in experimental animals⁴, including pulmonary oedema, congestion and haemorrhage, are not primary events, but instead represent secondary or tertiary pathological changes. 4-Ipomeanol may serve as a useful tool for the elucidation of pathophysiological events related to Clara cell damage as well as the normal physiological role of the Clara cell.

To determine whether metabolism of 4-ipomeanol was required to produce the tissue-bound radioactivity, animals were pretreated with piperonyl butoxide, an inhibitor of the metabolism of 4-ipomeanol by both hepatic and pulmonary mixed-function oxidases⁴, before administration of the radiolabelled toxin. Histological examination of the lungs from these animals revealed a striking decrease in covalently-bound radioactivity in the bronchiolar cells (Fig. 3*a, b*) and the total absence of bronchiolar necrosis (not shown), even though both the blood and the pulmonary concentrations of the unmetabolised toxin were markedly elevated by the pretreatment (Fig. 3*c*). This experiment further illustrates that a highly reactive metabolite of 4-ipomeanol, which binds covalently with the target cells and which can be localised by the technique described here, is responsible for the Clara cell necrosis.

In studies of the dose dependency of bronchiolar binding and necrosis by 4-ipomeanol, it was shown that the sensitivity to the toxin decreases as airway size increases. With minimally toxic doses, covalently-bound radioactivity was maximally concentrated in the smallest airways and the subsequent development of bronchiolar necrosis was restricted to those areas. As doses were increased, proportionately larger airways were also labelled and eventually showed bronchiolar cell necrosis. It is possible that the seemingly greater sensitivity of the smaller airways is related to the fact that the relative proportion of Clara cells to the less sensitive ciliated bronchiolar cells increases as airway size decreases³.

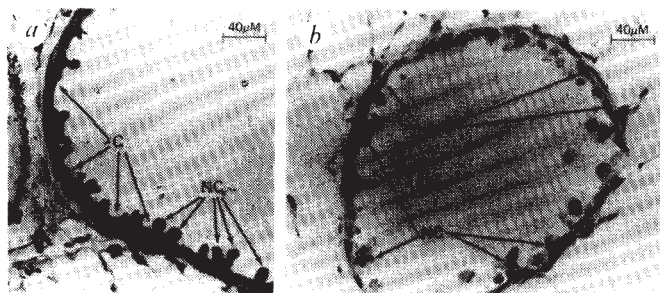


Fig. 2 *a*, Normal mouse bronchiole with well defined ciliated (C) and non-ciliated, or Clara (NC) cells. *b*, A typical pulmonary bronchiole from a mouse 16 h after i.p. administration of non-radiolabelled 4-ipomeanol (35 mg kg^{-1}). In *b*, the necrosis of Clara cells is apparent, and, although abnormal, ciliated cells still seem to be viable. Scale bars represent $40 \mu\text{m}$.

Previous attempts to localise pulmonary MFO *in vivo* have been inconclusive. Some investigators have suggested an alveolar cell locus⁶, and others have indicated that a bronchial cell localisation was likely⁷. One report indicated that pulmonary macrophages have little MFO activity⁸. Reid *et al.*⁹ found that bromobenzene, in addition to its toxic effects on the liver, also produces pulmonary bronchiolar necrosis that seems to be related to covalent binding to bronchioles of a reactive metabolite of the compound⁹. The reactive metabolite of bromobenzene, however, seems to be sufficiently stable to escape from its site of formation⁹. Thus, with this compound it was not possible to determine if the toxic metabolite binding to lung was formed in the liver or in the lung, or in both, and neither was it possible to determine the specific type of

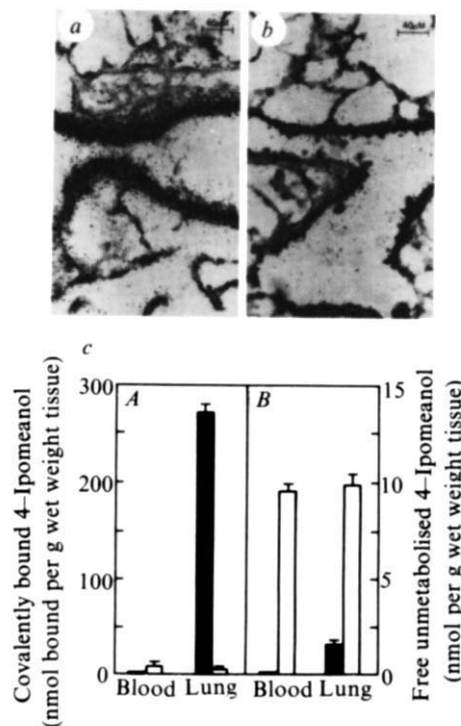


Fig. 3 Effect of piperonyl butoxide pretreatment ($1,600 \text{ mg kg}^{-1}$, i.p. 1 h previously) on bronchiolar alkylation (*a* and *b*) and on blood and pulmonary levels of covalently-bound (solid columns) and free 4-ipomeanol (open columns) (*c*) in the rat. All tissues were removed and processed 2 h after administration of the ^{14}C -labelled toxin (20 mg kg^{-1} , i.p.). Covalently-bound toxin was assayed as described in ref. 4; free 4-ipomeanol was isolated using procedures described in ref. 2 and quantitated by liquid scintillation counting. Data in frame *c* are means ($\pm \text{S.E.M.}$) from groups of seven animals each. *A*, Control rats; *B*, treated rats. Scale bars represent $40 \mu\text{m}$.

bronchiolar cell to which the metabolite was bound, and which type of cell eventually became necrotic.

The physiological role of the Clara cell is unknown. Some argue that it is a source of pulmonary surfactant, whereas others feel its primary role is as a participant in the 'mucociliary elevator'. Pulmonary anatomists, however, have speculated that based on morphological features the Clara cell is metabolically a very active cell, with secretory functions. It is known to be richly endowed with smooth endoplasmic reticulum (SER)³ and this may be significant in the light of the present investigations, as SER is associated with MFO-activity in other cells, such as hepatocytes¹⁰. It is also interesting that the covalently bound 4-ipomeanol observed in the present experiments is most heavily concentrated in the cytoplasmic cap of the Clara cells, which is also the principal location of the SER³; nuclear labelling is minimal (Fig. 1*f*).

In summary, these studies indicate the Clara cell is an important potential site of oxidative metabolism of xenobiotics in the lung. Pathological changes of Clara cells, such as necrosis, or neoplastic transformation, which are produced by MFO-activated chemicals may therefore be explainable on that basis. Moreover, since the Clara cell possesses enzymes required for metabolic activation of certain chemical carcinogens, it must be considered a prime candidate as a cell of origin for at least some bronchogenic cancers. In continuing studies we hope further to define the biochemical characteristics of normal Clara cells, and to compare these properties with those of pulmonary tumours. The identification of biochemical similarities within specific histological types of human bronchogenic carcinomas, may implicate specific cell(s) of origin, and could possibly have important therapeutic use. In a tissue of relatively heterogeneous cellular composition such as the lung, cell types with enzymes capable of activating specific carcinogens may give rise to tumour cell populations uniquely susceptible to cytotoxins activated by the same enzymes. The possible use of specific bronchiolar alkylating agents, with 4-ipomeanol as the

prototype, should be explored as a new approach to chemotherapy of bronchogenic cancer of the lung.

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Received 14 June; accepted 9 August 1977.

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Identification of a membrane-embedded segment of the large polypeptide chain of (Na⁺, K⁺)ATPase

The sodium pump or (Na⁺, K⁺)ATPase couples hydrolysis of ATP to the active transport of Na⁺ and K⁺ across the cell membrane. The firm association of the enzyme with the membrane allows its purification from the outer renal medulla by selective extraction of other membrane proteins using sodium dodecyl sulphate (SDS)¹. Two polypeptide components remain in the purified preparation of (Na⁺, K⁺)ATPase. A catalytic protein with molecular weight (MW) about 100,000 (100 K) which constitutes 60-70% of the total protein and a smaller sialoglycoprotein of unknown function^{2,3}. The arrangement of these proteins within the membrane is an important but poorly understood aspect of the Na pump structure. Presumably the catalytic regions of the enzyme are in contact with the aqueous phase at the membrane surface, while other portions of the protein are in intimate contact with the lipid and account for its firm anchorage. The lipid associated domains of the pump are of special interest because in addition to a purely structural role, they may also form parts of the ion conducting pathways. We have been able to identify presumptive intramembranous portions of the Na pump protein by labelling them covalently from within the membrane with 5-[¹²⁵I]-iodonaphthyl-1-azide (INA). This label is a very hydrophobic light-sensitive compound developed previously for this purpose and tested with other systems⁴⁻⁷. A segment(s) of approximate MW 12 K derived from the large polypeptide chain has been identified by combining the INA-labelling technique with extensive proteolysis of the membrane bound enzyme. Graded proteolysis of the labelled (Na⁺, K⁺)ATPase has also permitted an estimate of the position of this segment within the large chain.

In the dark INA is unreactive and partitions (> 98%) into membranes^{5,6}. Subsequent irradiation at 310 nm (ϵ 21,400) converts INA into a reactive nitrene which inserts covalently into the protein and lipid components of the (Na⁺, K⁺)ATPase preparation (Fig. 1a). The distribution of radioactivity between protein and lipid could be controlled by varying the concentration ratio of label to enzyme. At low INA-to-protein ratios (roughly 0.5 mol INA per mol of enzyme) the label was attached almost exclusively to the large chain (Fig. 1a). In this experiment inhibition of the (Na⁺, K⁺)ATPase activity was less than 10%. The sialoglycoprotein was barely labelled and less than 20% of the radioactivity was associated with the lipid. Evidence for a membrane-bound segment of the large

chain was obtained by extensive digestion of labelled (Na⁺, K⁺)ATPase with trypsin (Fig. 1b) or thermolysin (not shown). In either case radioactivity was transferred essentially quantitatively from the large chain to a fragment(s) of MW about 12 K. After the extensive proteolysis only 45-50% of the original protein remains in the membrane whereas less than 10% of the radioactivity could be released from the membrane by

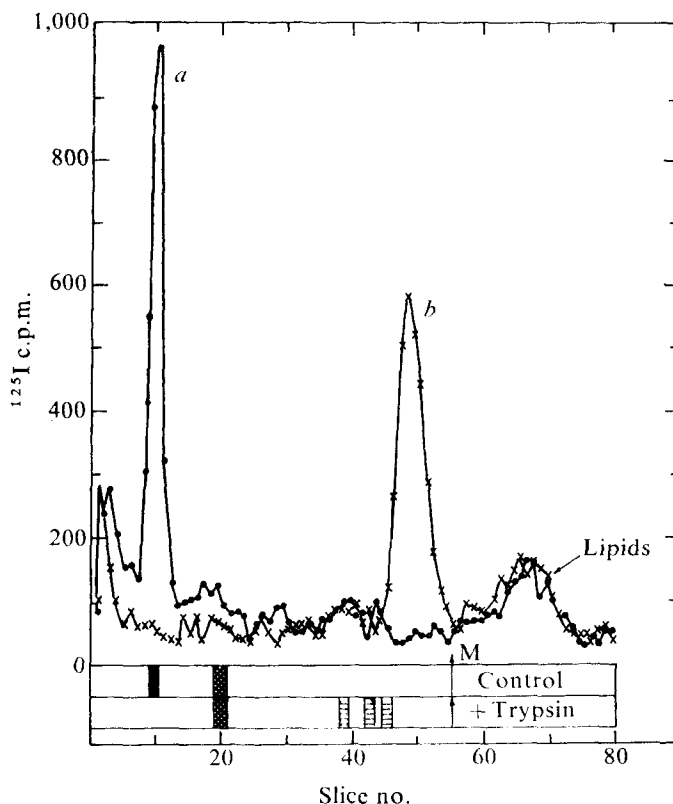


Fig. 1 Labelling of (Na⁺, K⁺) dependent ATPase with 5-[¹²⁵I]-iodonaphthyl-1-azide and effect of extensive trypsinolysis of labelled membranes. 0.6 mg of (Na⁺, K⁺)ATPase prepared from pig kidney¹ (specific activity 32.2 IU per mg protein) was suspended in 1 ml of a medium containing histidine, 25 mM, pH 7.5; EDTA, 1 mM and NaCl 100 mM. 5-[¹²⁵I]-iodonaphthyl-1-azide (0.2-0.3 µg of material with SA 1-3 × 10⁸ c.p.m. per µmol INA) prepared by methods described in ref. 7, was added in the dark in 10 µl of ethanolic solution. The membranes plus label were incubated at 37 °C for 5 min in the dark to allow full partitioning of the INA into the membranes. The suspension was then irradiated for 2 min with light mainly of wavelengths 313 and 365 nm produced by a mercury lamp HB200. The mercury lines below 300 and above 400 nm were excluded by passing the light through a Corning 7-60 band pass filter and Schott WG 305 cut off filter. After irradiation a small sample of the membranes was removed for measurement of (Na⁺, K⁺)ATPase activity¹⁰ and the rest was dialysed overnight at 0 °C against 1 l of solution containing histidine 25 mM, pH 7.5; EDTA 1 mM; bovine serum albumin 1 mg ml⁻¹. Radioactive diffusible byproducts of INA removed by this procedure amounted to about half of the total radioactivity added initially. For extensive proteolysis, portions of dialysed labelled enzyme containing 100 µg of protein were incubated for 1 h at 37 °C in 120 µl of medium containing imidazole 25 mM, pH 7.5, KCl 150 mM, CaCl₂, 1 mM, and trypsin 10 µg Sigma Type III. Then 1 ml of the medium (minus trypsin) was added to trypsinised and control membranes and these were centrifuged at 40,000 r.p.m. for 1 h in a Beckman type 65 rotor. About 10% of the total radioactivity was found in the supernatant, but there was no significant difference between the control and trypsinised enzyme. The pellets were dissolved in 100 µl SDS 2%, mercaptoethanol 1% and the dissolved material electrophoresed on polyacrylamide gels in SDS essentially as described in ref. 10. After staining with Coomassie blue and destaining in methanol acetic acid (see ref. 10) which also removed excess radioactive byproducts, the gels were cut into slices 1 mm thick, and the ¹²⁵I counted. The figure shows the distribution of radioactivity and position of Coomassie blue staining bands in duplicate gels. The arrows and letter M mark the tracker dye front. a, Control; b, following extensive trypsin digestion.

centrifugation. The large polypeptide chain disappeared and peptides with MW 58 K, 23 K, 20 K and 17 K staining with Commassie blue were observed; staining in the region containing the radioactivity was weak or absent (see Fig. 1). The specificities of trypsin and thermolysin are very different and so it is quite likely that undigested peptides are protected from complete proteolysis by their association with the lipids.

After exposure of the enzyme at a much higher concentration of INA (namely 25–50 mol INA per mol of enzyme) the lipids became labelled to a level 6–8-fold greater than the protein and incorporation was also seen in the glycoprotein. In these conditions the $(\text{Na}^+, \text{K}^+)\text{ATPase}$ activity was almost completely inhibited and radioactivity equivalent to roughly one molecule of INA was incorporated into the large chain per mol of enzyme. Exhaustive trypsinisation of these membranes resulted in essentially the same pattern of the label distribution as shown in Fig. 1b.

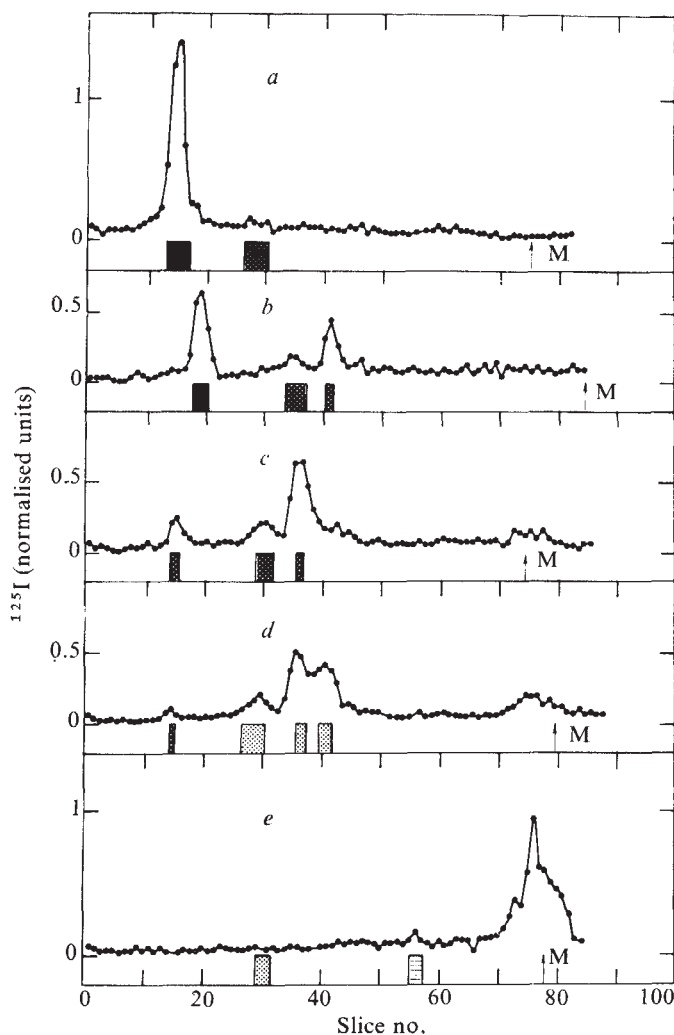


Fig. 2 Distribution of radioactivity in fragments produced by controlled trypsinolysis in K^+ medium of INA labelled $(\text{Na}^+, \text{K}^+)\text{ATPase}$. $(\text{Na}^+, \text{K}^+)\text{ATPase}$ was labelled and dialysed as in Fig. 1. Aliquots containing 50–75 μg were incubated with trypsin at 37°C in a total volume of 0.5 ml in the following conditions: trypsin $\sim 3 \mu\text{g}$; KCl , 150 mM; imidazole 17 mM, pH 7.5; CaCl_2 , 1 mM. In b, CaCl_2 was absent and sample e contained 10 μg of trypsin. Trypsin inhibitor (0.5 ml of $20 \mu\text{g ml}^{-1}$) was added to the control sample (a) before addition of trypsin, and to the other samples after incubation with trypsin for the following times: b, 10 min; c, 15 min; d, 40 min and e, 60 min. The samples were then taken through the same steps as in Fig. 1. In these experiments the gels were run for 8–9 cm to facilitate observation of intermediate radioactive peaks. The lipids have migrated out of the gel into the lower bathing solution. Units of radioactivity have been normalised to account for variations in the total amount of protein applied to the gels in the different samples.

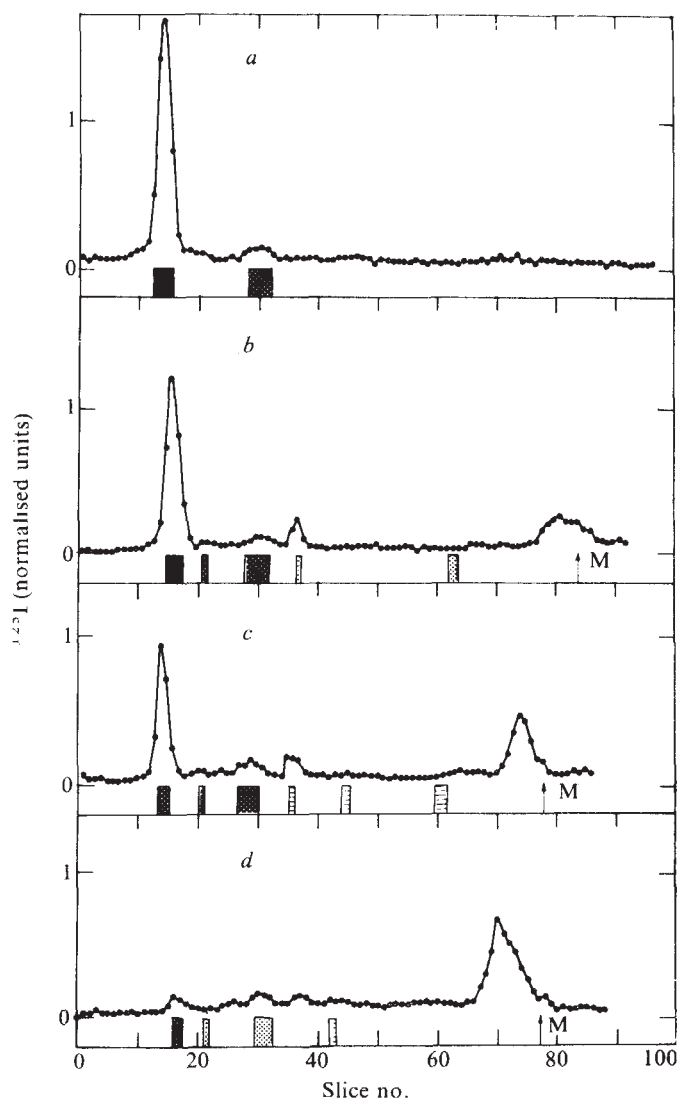


Fig. 3 Distribution of radioactivity in fragments produced by controlled trypsinolysis in a Na^+ medium of INA labelled $(\text{Na}^+, \text{K}^+)\text{ATPase}$. $(\text{Na}^+, \text{K}^+)\text{ATPase}$ was labelled and dialysed as in Fig. 1. Aliquots containing 50–75 μg were incubated with trypsin at 37°C in a total volume of 0.5 ml in the following conditions: trypsin, 3 μg ; NaCl , 150 mM and imidazole 17 mM, pH 7.5. Sample e also contained CaCl_2 1 mM. 0.5 ml of trypsin inhibitor $20 \mu\text{g ml}^{-1}$ was added to the control a before addition of trypsin and to the other samples after the following incubation times; b, 20 min; c, 40 min and d, 60 min. All other procedures were as in Figs 1 and 2.

The preferential insertion into the large chain at low concentrations of INA and inhibition of the $(\text{Na}^+, \text{K}^+)\text{ATPase}$ could imply contrary to expectation, that the label binds specifically to catalytic areas of the protein. Such areas are often hydrophobic clefts in enzyme surfaces. This possibility is unlikely for the following reasons. All catalytic functions are destroyed by relatively limited proteolysis^{8,9} but, as seen above, exhaustive proteolysis did not release the label. Furthermore, binding to the ATP binding site of a fluorescent ATP analogue, formycin triphosphate¹¹ was unaffected by incorporation of sufficient idonaphthyl groups to inhibit activity by 70%, and ATP did not alter incorporation of label into the protein when it was present during irradiation (not shown). We did not study the mechanism of inhibition any further. But, the observation that INA does not interfere with ATP binding is compatible with the notion that it may inhibit catalysis by perturbing protein–lipid interactions, since it has been shown that ATP binding is unaffected after inactivation of $(\text{Na}^+, \text{K}^+)\text{ATPase}$ by complete delipidation¹².

We have examined labelling of the large protein in Na^+ - or K^+ -containing media. The enzyme adopts specific Na^+ - or K^+ -dependent conformations^{8,12-14} and so it was interesting to ask whether these different states are accompanied by a change in accessibility of the protein to INA in the bilayer. In six separate experiments with different concentration ratios of label to protein we observed 10–25% greater incorporation into the large chain in the presence of KCl than with NaCl. This may mean that the conformational change following binding of K^+ leads to an altered relationship of protein to lipid.

In attempts to localise the 12 K fragment we exploited the fact that major fragments of the large chain can be produced by controlled tryptic cleavage in the presence of NaCl or KCl because the conformational response of the protein to cation binding is reflected in exposure of different bonds to tryptic attack^{8,9}. Thus, fragments with MW 58 K and 46 K were formed by primary cleavage of the large chain in presence of KCl. A fragment of 78 K was seen after digestion in the presence of NaCl. The distribution of INA in protein fragments generated by controlled trypsinolysis of enzyme labelled at low INA concentrations is shown in Figs 2 and 3. These results show clearly that the binding of INA does not prevent the conformational response of the large chain to Na^+ and K^+ . In the presence of KCl (Fig. 2) most of the radioactivity of the large chain was transferred to the 46 K fragment, but some labelling of the 58 K fragment was also seen. Comparison of radioactivity in the peaks with the content of protein obtained from absorbance scans of the gels showed that about 80% of the label was transferred to the 46 K fragment and only 20% to the 58 K fragment. Subsequently a 36 K fragment and the terminal 12 K peptide appear. The 46 K and the 36 K fragments therefore include segments which are embedded in the bilayer. The low concentration of label in the 58 K fragment may be due either to labelling of different parts of the large chain or to variation of the position of the tryptic split within the large chains in the preparation.

In the presence of NaCl (Fig. 3), the distribution of label was much more distinct. The 78 K fragment was completely devoid of radioactivity and the terminal 12 K fragment seems to arise almost by primary cleavage of the large chain. A minor fraction of the label, at most 2%, was found in a 37 K fragment. This implies that the radioactive 12 K segment or segments are localised near one end of the large chains which give rise to the 78 K fragment in the presence of NaCl.

Our results support previous evidence that idonaphthylazide acts as a hydrophobic probe which labels membrane proteins from within the bilayer. The selectivity of the label for a small segment of the large peptide does not imply that other regions of the protein are not embedded in the membrane, for the labelled segment may hinder access of the label to other areas of the large peptide or indeed to the glycoprotein component. Preferential binding to this segment, or kinetic factors could explain the discrimination against unlabelled regions of protein and lipid side chains. Our work illustrates that combining specific labelling from within the lipid core with proteolytic digestion at the membrane surface may be an important tool for identifying segments of membrane proteins in intimate contact with the lipids.

We thank Dr T. Bercovici for ¹²⁵I-INA and Mrs Rivka Goldshlegger and Annie Sloth for excellent technical assistance. P.L.J. was a recipient of a short-term fellowship from EMBO.

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Received 7 July; accepted 12 August 1977.

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Synthesis of haemoglobin Wayne in erythroid cells

HETEROZYGOES for haemoglobin (Hb) Wayne possess two minor haemoglobin components that migrate more rapidly than HbA on electrophoresis at pH 8.6. Each of the minor haemoglobin components contains an abnormal α chain in which the carboxyl-terminal tripeptide sequence, Lys-Tyr-Arg, has been replaced by an octapeptide¹. The slower of the two components, henceforth designated Wayne-Asn, has the following octapeptide sequence: Asn-Thr-Val-Lys-Leu-Glu-Pro-Arg, whereas the faster one (Wayne-Asp) has exactly the same sequence except that asparagine at position 139 is replaced by aspartic acid. (These components, formerly designated¹ Wayne-1 and Wayne-2, respectively, have been renamed for clarity.) This is the first variant described in which deamidation of the gene product is believed to occur. The purpose of this study was to demonstrate the proposed deamidation and to explain another interesting feature of the Hb Wayne phenotype in heterozygotes: namely, the presence of the variant haemoglobins in markedly reduced quantities relative to HbA (3% and 4%, respectively, for Hb Wayne-Asn and Hb Wayne-Asp).

Peripheral blood red cells from two heterozygotes with Hb Wayne and bone marrow cells from one of them were incubated for 60 min in the presence of ³H-labelled leucine. A fraction of the labelled haemolysates was converted into globin immediately by precipitation in acid-acetone. The α/β ratios obtained, based on total counts and the specific activities, indicate that the synthesis of α chains is equal to that of the β chains in the peripheral blood and bone marrow (balanced synthesis). Moreover, a free α chain peak could not be demonstrated after chromatography of the haemolysates on Sephadex G-100 gel filtration columns.

In order to compare more precisely the specific activities of the various α chains (Table 1), it was necessary to first purify the haemoglobins in the labelled haemolysates by column chromatography of the haemolysates on carboxymethyl cellulose². This was followed by preparative isoelectric focusing of the peaks containing the Hb Wayne fractions using LKB columns and finally globin chain separation³. The Wayne chain fractions obtained were shown to be free of contamination by peptide chromatography of tryptic digests.

In the peripheral blood incubations the amount of radioactivity corresponding to the α^{Wayne} chains was less than 11% of the total α chain radioactivity (Table 1). The corresponding value in the bone marrow was 11.4%. These figures were considerably lower than the 25% expected on the basis of the two loci believed to be present for α chains. The $\alpha^{\text{Wayne-Asn}}/\alpha^{\text{A}}$ specific activity ratio was 2.40 to 2.46 in the peripheral blood and 3.1 in the bone marrow, suggesting instability of the $\alpha^{\text{Wayne-Asn}}$ chains. In both peripheral blood and bone marrow, the specific activity of the $\alpha^{\text{Wayne-Asp}}$ chains was lower than that of the $\alpha^{\text{Wayne-Asn}}$ chains (Table 1). Time course experiments using peripheral blood showed no change in the $\alpha^{\text{Wayne-Asn}}/\alpha^{\text{Wayne-Asp}}$ ratio from 4 min to 3.5 h (not shown).

In order to evaluate the difference in labelling of the two types of variant α chains a pulse-chase experiment was performed. Peripheral blood red cells from a heterozygote were incubated in the presence of ³H-leucine for 15 min (pulse) after which they were washed and a portion was immediately frozen. The remaining cells

Table 1 Incorporation of ^3H -leucine into α and β chains following incubation of red cells for 60 min

	α/β ratios of globin from crude haemolysates		Radioactivity of purified α chains					
	Total counts	Specific activity	Specific activity (c.p.m. per mg)		% Of total α chain radioactivity			
			$\alpha^{\text{Wayne-Asn}}$	$\alpha^{\text{Wayne-Asp}}$	α^{A}	$\alpha^{\text{Wayne-Asn}}$	$\alpha^{\text{Wayne-Asp}}$	α^{A}
Peripheral blood (WS)	0.97	0.94	644	115	262	8.36	1.80	89.84
Peripheral blood (GS)	1.04	0.96	968	202	403	6.96	1.95	91.09
Bone marrow	1.12	0.91	40,039	837	12,880	11.16	0.27	88.57

A fraction of the crude haemolysates was converted to globin immediately by precipitation in acid acetone. The globin obtained was chromatographed on columns of Sephadex G-100 in 20% HCOOH^{15} . After chromatography globin fractions were recovered by freeze drying. Another fraction of the haemolysate was used for purification of the various haemoglobin components for accurate determination of radioactivity in the α^{Wayne} chains. Globin chain separation was carried out according to Clegg *et al.*³ Aliquots of 1 ml of each fraction were added to 10 ml Instagel (Packard) and counted by scintillation spectroscopy. The total radioactivity under each peak was summated. The various chain components were then pooled, desalted on Biogel P2 columns equilibrated with 0.5% formic acid, and lyophilised. The chains were re-dissolved in distilled water and the protein concentration determined by the Lowry method²⁹.

were reincubated in a medium containing unlabelled leucine (chase) for 1, 4 and 6 h. The specific activities of the α^{A} and β chains remained unchanged during the chase period as did that of the $\alpha^{\text{Wayne-Asn}}$ and $\alpha^{\text{Wayne-Asp}}$ chains (Table 2), suggesting that during the short period of the chase no detectable conversion or degradation of the abnormal chains relative to each other or to α^{A} occurs. If deamidation is a slow process, then a greater proportion of Hb Wayne-Asn relative to Hb Wayne-Asp should be present in reticulocytes when compared with the more mature red cells. A reticulocyte fraction was prepared from the peripheral blood of a heterozygote by means of a discontinuous Ficoll density gradient⁴. Isoelectric focusing in polyacrylamide gels of the reticulocyte haemolysate revealed a markedly decreased proportion of Hb Wayne-Asp when compared to the haemolysate prepared from unfractionated peripheral blood. Moreover, the proportion of Hb Wayne-Asn in reticulocytes was approximately twice as much as in peripheral blood (Fig. 1).

The above data support the hypothesis that $\alpha^{\text{Wayne-Asn}}$ chains are synthesised initially and subsequently converted to $\alpha^{\text{Wayne-Asp}}$ chains. Robinson and others⁵⁻⁸ have shown that specific glutamyl and asparaginyl residues have characteristic rates of deamidation that are determined by the nature of neighbouring amino acid residues in both the primary and tertiary structures. The discovery of Hb Providence, a β chain variant present as two components, one having asparagine instead of lysine at position 82 and the other having aspartic acid at the same position, indicates that deamidation is not unique to Hb Wayne⁹⁻¹⁰. It is also possible that Hb J-Singapore¹¹ is not a double substitution variant but a neutral substitution at α^{79} Ala $\rightarrow$ Gly followed by deamidation at α^{78} to yield aspartic acid¹².

It is interesting to note that $\alpha^{\text{Wayne-Asp}}$ chains account for a higher proportion of the total α -chain radioactivity in peripheral blood than in bone marrow (Table 1); the $\alpha^{\text{Wayne-Asn}}/\alpha^{\text{Wayne-Asp}}$ ratio was 0.5 in the former and 0.06 in the latter. A possible explanation for all of these findings is that the asparagine at residue 139 of $\alpha^{\text{Wayne-Asn}}$ forms a cyclic imide, as proposed by Bornstein¹³, while the chains are in the nascent chain or monomer conformation. While in this conformation the cyclic imide is rapidly hydrolysed to the aspartyl form. Once the $\alpha^{\text{Wayne-Asn}}$ chain is incorporated into the Hb Wayne tetramer further cyclisation is blocked by steric

hindrance but those cyclised chains that had formed are hydrolysed very slowly. In the bone marrow any $\alpha^{\text{Wayne-Asp}}$ monomer chains produced by deamidation of $\alpha^{\text{Wayne-Asn}}$ monomers might be rapidly degraded by proteolytic enzymes that are not active in mature erythrocytes^{14,15}.

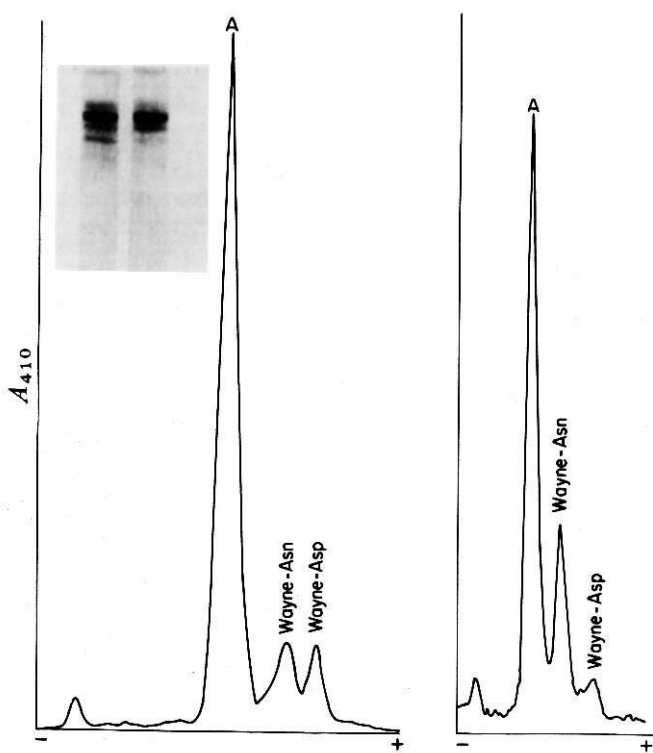


Fig. 1 Isoelectric focusing in polyacrylamide gels of peripheral blood haemolysate (left) and reticulocyte haemolysate (right). The densitometric tracings were made on unstained gels at 410 nm using a Gilford recording spectrophotometer.

Table 2 α Chain specific activities during a pulse chase experiment

Incubation time	Specific activity (c.p.m. per mg)		$\frac{\alpha^{\text{Wayne-Asp}}}{\alpha^{\text{A}}}$	$\frac{\alpha^{\text{Wayne-Asp}}}{\alpha^{\text{A}}}$	$\frac{\alpha^{\text{Wayne-Asn}}}{\alpha^{\text{Wayne-Asp}}}$
	$\alpha^{\text{Wayne-Asn}}$	$\alpha^{\text{Wayne-Asp}}$			
15 min pulse	1,510	275	1.80	0.33	5.49
1 h chase	1,631	286	1.84	0.32	5.70
4 h chase	1,572	283	1.73	0.31	5.55
6 h chase	1,590	269	1.79	0.30	5.91

The presence of Hbs Wayne as minor components is shared by Hbs Constant Spring¹⁶⁻¹⁸, Koya Dora¹⁹, Icaria²⁰ and Seal Rock²¹, other variants characterised by elongated chains. Because the α^{Wayne} chains comprise only 11% of the total α chains synthesised during the period of incubation, instability of the chains does not fully account for the low proportion of Hb Wayne. A delay in translation of the α^{Wayne} chains as a result of a deficiency of tRNA for one or more of the amino acids corresponding to the abnormal sequence cannot be excluded, although such a delay has not been found in any of the abnormal haemoglobins that are present in reduced amount and that have been studied²²⁻²⁵, including Hb Constant Spring¹⁶. Perturbation of termination of translation *per se* seems also an unlikely explanation since the synthesis of Hb Cranston, a frameshift mutant of the β chain, is not so severely impaired²⁶. Yet another possible explanation, that the mRNA has been rendered unstable by the deletion responsible for the frame shift, has been excluded by the demonstration that no more Hb Wayne components are synthesised in bone marrow than in peripheral reticulocytes.

The expectation that an α -chain mutant would direct the synthesis of 25% of the α -chain mRNA is based on the unproven assumption that there are two loci that are equally active in directing α -chain synthesis. This assumption is partly based on the fact that individuals heterozygous for Hbs J-Buda and G-Pest²⁷ possess close to 25% of these variants, along with approximately 50% of Hb A, as well as the fact that a large number of other α -chain structural mutants comprise 20-25% of the haemoglobin of heterozygotes. Haemoglobin synthesis studies have not been performed on most of these variants. Consequently their true rate of synthesis is not known. Therefore, the reduced production of the Constant Spring and α^{Wayne} chains could also be explained on the basis of multiple α -chain loci that are not equally active in α -chain synthesis. Such a hypothesis has been invoked to explain a greater than expected amount of Hb J-Mexico in heterozygotes²⁸. It is possible to envision one major locus, another of intermediate activity, and a third locus accounting for only a minor proportion of alpha chains. The mutation in α^{Wayne} would correspond to the intermediate locus and that of $\alpha^{\text{Constant Spring}}$ would correspond to the minor locus. A complete understanding of these complex abnormalities requires study of additional informative mutants.

This work was supported in part by USPHS grants GM15419 and NIH 5-T32-01723.

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Received 18 July; accepted 10 August 1977.

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Red cell charge is not a function of cell age

REPORTS^{1,2} that the oldest circulating human erythrocytes have an electrophoretic mobility up to 30% lower than the youngest cells have been widely accepted, particularly since such findings suggest a plausible mechanism for the removal of the oldest cells from circulation. For those studies^{1,2} the red cells were separated on the basis of age by using the accepted relationship that older cells are denser on the average than younger cells. We report here that electrophoretic mobility studies conducted independently by conventional electrophoresis, streak width measurements and electrophoretic light scattering have shown the mobilities to be the same for red cell fractions of differing density and hence age *in vivo*. These data suggest that hypotheses which invoke a role for decreasing surface charge density in the mechanism of senescent red cell recognition *in vivo* are unsound.

The non-random nature of the red cell elimination process has led to suggestions that membrane determinants such as red cell surface charge³ and/or antibody binding to the cell surface⁴ may provide specificity as a result of age-dependent alterations which enhance cell adhesion to phagocytic cells.

The observation that various sialoglycoproteins are rapidly eliminated from the blood following removal of their sialic acid⁵ has prompted considerable speculation on the role of sialic acid loss in the ageing and elimination of red cells^{6,7}. In some instances, normal lifespans have been observed for sialic acid deficient red cells⁸⁻¹⁰. But, the sialic acid content of old (dense) red cells is lower than young (less dense) cells¹¹⁻¹³. As the major source of negative surface charge on human red cells is the carboxyl group of membrane-bound *N*-acetylneuraminic acid¹⁴, an age-related decrease in electrophoretic mobility^{1,2} is consistent with a net loss of sialic acid per unit of membrane surface area.

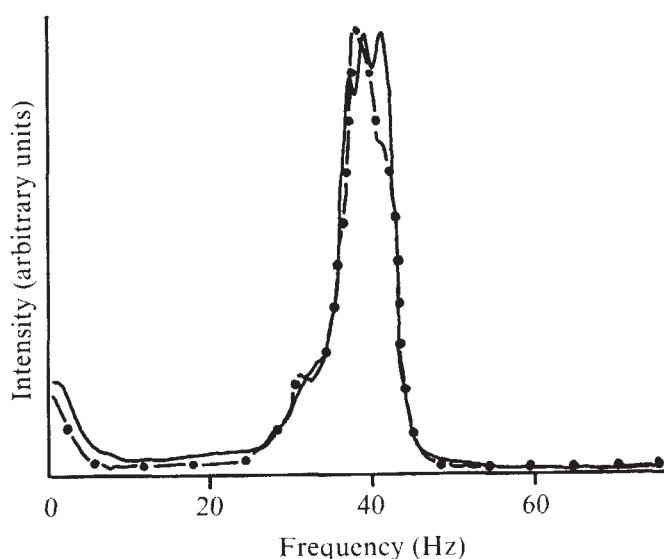


Fig. 1 Electrophoretic light scattering spectra¹⁹ for least dense (top, —) and most dense (bottom, ---) human red cell subpopulations at 25 °C in 0.0145 M NaCl-4.5% w/v sorbitol-0.007 M bis-Tris buffer at pH 7.3. The top and bottom fractions each representing ~2% of the whole population were obtained by centrifugation of the cells in their own plasma for 30 min, 140,000g at 4 °C followed by three washes in the electrophoresis buffer. The mean Doppler shift for both spectra is 39.5 Hz, which corresponds to an electrophoretic mobility of $2.75 \pm 0.05 \mu\text{m}^{-1} \text{V}^{-1} \text{cm}$ after a correction for electro-osmosis (9.8 Hz). Experimental parameters were: scattering angle, 14.9° ; laser wavelength, 514.5 nm; electric field, 16.2 V cm^{-1} and frequency resolution of ~0.03 Hz. In these conditions a 20% difference in mobility would produce a difference in shift frequency of ~6 Hz. The spectra shown for the subpopulations were indistinguishable from that of the whole population (not shown).

Table 1 Streak width and aspartate aminotransferase activities¹⁷ for whole red cell populations and extreme density fractions representing 2–7% of the whole populations

Sample no.	Aspartate aminotransferase (IU per 10 ¹⁰ RBC)			Electrophoretic streak width (mm)			
	Unfractionated	Top	Bottom	Unfractionated	Top	Bottom	Top and bottom
1	0.95	1.66	0.40	0.79	1.27	1.01	0.88
2	1.88	7.15	0.87	0.61	0.56	0.64	0.66
3	2.96	5.22	0.95	0.84	1.00	0.97	1.16
4	0.87	2.12	0.58	0.57	0.68	0.62	0.74
5	1.53	1.26	0.49	0.62	0.64	0.67	0.60
5a	1.53	1.13	0.58	0.62	0.62	0.64	0.62
6	1.20	1.79	0.89	0.79	0.98	0.81	1.07
6a	1.20	1.60	1.02	0.79	0.78	0.79	0.80

Samples 1, 4, 5 and 6 were obtained from adults, samples 2 and 3 from the umbilical cord of infants at birth. All samples were fractionated by repeated centrifugation for 30 min at 2000g on phthalate ester mixtures¹⁸ except for samples 5a and 6a which were obtained by ultracentrifugation at 50,000g, 20 °C for 2 h. Cell suspensions (2% v/v) in pH 7.4, 16.5 mM Tris-acetate buffer containing 5% sucrose were electrophoresed at 21 °C, 80 V cm⁻¹. Streak widths are to be compared with the 13.8 mm total distance of migration for a mobility of 2.3 $\mu\text{m s}^{-1} \text{V}^{-1} \text{cm}$ RBC, red blood cell.

To detect any significant dispersity in electrophoretic mobility which accompanies changes in density and *in vivo* ageing of red cells, the extreme ends of the red cell density distribution were examined for evidence of differences in electrophoretic mobility. Using various red cell density fractionation procedures, studies conducted independently in our three laboratories with different instrumentation and conditions do not show any experimentally significant electrophoretic mobility differences for the extreme red cell subpopulations.

Electrophoresis of erythrocyte fractions by the continuous-flow streak deflection method¹⁵ was carried out in the conditions previously reported¹⁶ using the Kolin endless belt electrophoresis apparatus. If present, heterogeneity in electrophoretic mobility of cell populations is evidenced by progressive sample band broadening on passage through the electrical field of the instrument and by actual band splitting for samples with multimodal mobility distributions. Examination of the banding behaviour for whole and density-fractionated human red cell populations (Table 1) gave no indication of electrophoretic inhomogeneity. The maximum observed bandwidths were consistent with about a 5% range in electrophoretic mobility for mixtures of cells made from the density extremes. The band or streak width expected on the basis of Yaari's data² for 'top' and 'bottom' cell mixtures is about five times the observed value. Furthermore the band should have shown signs of observable splitting as the run progressed. Marked

differences in red cell aspartate aminotransferase activity between the fractions indicated that significant cell separation according to cell age had been achieved¹⁷.

Electrophoretic light scattering spectra (Fig. 1) for 'top' and 'bottom' fractions of red cells obtained by ultracentrifugation also failed to show significant differences between the mean mobilities or the mobility distributions.

Analytical electrophoretic mobility data for samples of top and bottom cell populations obtained by two different density fractionation techniques also showed no differences either at high or low ionic strength (Table 2). Extreme subpopulations representing as little as 1% (not shown) were not found to differ.

In each of the described electrophoresis systems the expected mobility differences² for red cells from the extremes of the density distribution should have been detected with ease. We conclude from the lack of any clear indications of mobility heterogeneity in these studies which involved different density separation and electrophoretic monitoring techniques that no measurable differences in mobility are correlated with red cell density through the central ~95% of the density distribution. This is in contrast to the observations of Yaari² which showed electrophoretic mobility to be a monotonic function of cell density through this range.

Electrophoretic mobility is recognised as a measure of net surface charge density²³. A constant electrophoretic mobility for red cells of different densities implies that the effective net number

Table 2 Mobility data and descriptive indices for human red cells fractionated with phthalate esters¹⁸ and by high-speed centrifugation²⁰

Subpopulation	MCHC (g per 100 ml)	NANA (fg per cell)	GOT (IU per cell $\times 10^{11}$)	Electrophoretic mobility ($\mu\text{m s}^{-1} \text{V}^{-1} \text{cm}$)	
				0.15 M NaCl	0.03 M NaCl
Phthalate ester					
<i>a</i> Top 6%	32.8	20.1	—	$-1.10 \pm 0.06(30)$	$-1.72 \pm 0.11(56)$
Bottom 10%	40.6	18.3	—	$-1.10 \pm 0.06(30)$	$-1.72 \pm 0.09(46)$
<i>b</i> Top 5%	35.8	20.8	16.9	$-1.09 \pm 0.07(60)$	$-1.71 \pm 0.09(50)$
Bottom 6%	41.4	19.6	6.6	$-1.11 \pm 0.05(32)$	$-1.72 \pm 0.09(28)$
<i>c</i> Top 7%	34.0	21.2	13.5	$-1.09 \pm 0.07(20)$	$-1.74 \pm 0.06(20)$
Bottom 2%	39.9	16.8	4.9	$-1.12 \pm 0.08(20)$	$-1.73 \pm 0.12(20)$
Murphy fractionation					
<i>a</i> Top 5%	33.4	18.4	9.4	$-1.10 \pm 0.07(20)$	$-1.82 \pm 0.07(20)$
Bottom 5%	40.1	16.6	4.3	$-1.11 \pm 0.07(20)$	$-1.84 \pm 0.09(20)$
<i>b</i> Top 5%	31.4	17.8	10.7	$-1.08 \pm 0.04(20)$	$-1.77 \pm 0.10(20)$
Bottom 5%	35.8	15.9	2.0	$-1.10 \pm 0.06(28)$	$-1.78 \pm 0.07(20)$
<i>c</i> Top 5%	34.3	17.8	9.7	$-1.05 \pm 0.08(20)$	$-1.72 \pm 0.09(20)$
Bottom 5%	41.7	16.2	4.8	$-1.07 \pm 0.08(20)$	$-1.70 \pm 0.09(20)$
Pooled ratios	0.843	1.13	2.98	0.985	0.999
Top/bottom	± 0.026	± 0.07	± 1.36	± 0.009	± 0.008

Whole blood samples were collected by venipuncture from four adult subjects. Fractions were washed once in 2–4 volumes of autologous plasma, three times in > 20 volumes of pH 7.4, 0.144 M NaCl–0.01 M potassium phosphate (PBS). Mean cell haemoglobin concentration (MCHC) was calculated from haemoglobin assayed as cyanmethaemoglobin and microhaematocrit values after centrifugation for 5 min at 15,000g *N*-acetylneuraminic acid (NANA) was assayed in supernatants²¹ after incubation of $\sim 2 \times 10^9$ RBC per ml in PBS plus ~ 100 Behringwerke units of *Vibrio cholera* neuraminidase for 1 h at 37 °C. Aspartate aminotransferase (glutamic oxaloacetic transaminase, GOT) was assayed spectrophotometrically²² and activity is expressed in international units (IU) where one unit represents the conversion of 1 μmol of NADH to NAD⁺ in 1 min at 25 °C. Mobilities were measured at 25 °C in cylindrical chambers equipped with Ag/AgCl electrodes²³ or Pt electrodes²⁴, and operated at voltage gradients < 5 V cm⁻¹ and currents < 3 mA to minimise joule heating. Mean mobilities and standard deviations are given for the number of cells in parentheses. The two ionic strength media listed were buffered to pH 7.2 $\pm$ 0.2 with NaHCO₃ and 0.03 M NaCl contained 4.0% (w/v) sorbitol to maintain medium tonicity.

of negative charges per unit area of membrane surface as observed by electrophoresis remains constant even though the total number of charge groups, such as sialic acid, on the cell surface may be decreased as, for example, by membrane loss.

This work was supported by USPHS grants CA 13955 and HL 18284 and NSF grant PCM 72-05133. J.Y.J. was a Killam Postdoctoral Research Fellow. B.R.W. is an Alfred P. Sloan Research Foundation Fellow. We thank Frank Nordt and Bonnie Voyda for technical assistance.

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Ion-induced changes in head group conformation of lecithin bilayers

THE structure and orientation of the phosphocholine polar group in lecithin-containing membranes has been discussed extensively. Studies of egg-yolk lecithin and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), using neutron diffraction and ^2H and ^{31}P nuclear magnetic resonance (NMR), have led to the conclusion that the phosphocholine dipole is orientated parallel to the membrane surface¹⁻⁴. On the other hand, ^1H and ^{31}P NMR spectra of sonicated DPPC vesicles in the presence of trivalent ions suggested that the choline group is extended approximately perpendicular⁵ or folded parallel⁶ to the bilayer surface. We have, therefore, repeated our previous ^2H and ^{31}P NMR measurements in the presence of trivalent ions and report here that the addition of these shift reagents induces large changes in the spectral parameters, that is, the ^2H quadrupole splittings and the ^{31}P chemical shift anisotropy. These observations can only be interpreted in terms of specific ion-induced changes in the choline head group conformation of DPPC.

Typical spectral changes observed on addition of EuCl_3 , LaCl_3 and $\text{K}_3\text{Fe}(\text{CN})_6$ to unsonicated dispersions of DPPC are shown in Figs 1-3. The observed changes cannot

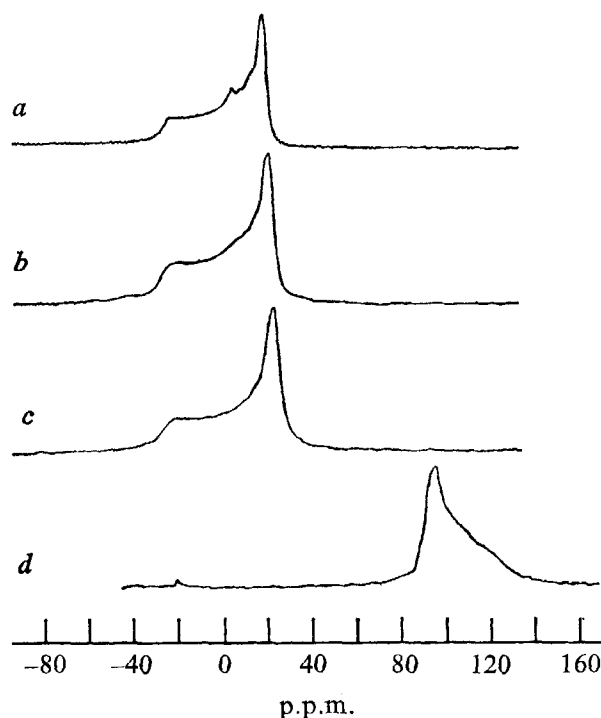


Fig. 1 Phosphorus-31 NMR spectra of unsonicated multibilayers of DPPC complexed with trivalent ions at 48 °C. *a*, No ions; *b*, $\text{Fe}(\text{CN})_6^{3-}$; *c*, $\text{La}(\text{III})$; *d*, $\text{Eu}(\text{III})$. Solutions of $\text{K}_3\text{Fe}(\text{CN})_6$, LaCl_3 , and EuCl_3 were prepared as described in ref. 5. Approximately 200 mg of DPPC were mixed with 0.5 M ion solutions to yield a final concentration of 50 wt % H_2O and a DPPC to ion mole ratio of 2.7. The samples were then sealed in glass ampoules and placed in 10-mm NMR tubes. The NMR spectra were obtained in the pulsed Fourier transform mode at 36.4 MHz using a Bruker HX-90 spectrometer/B-NC-12 data system equipped with a deuterium lock and home-built quadrature phase detection. Approximately 5,000 free induction decays were averaged to obtain these spectra. A proton-decoupling field of 10 W tuned to the CH_2 resonance of the choline head group moiety was applied. The chemical shifts are relative to 85% orthophosphoric acid. The residual chemical shift anisotropy ($\Delta\sigma$) is given approximately by the separation between the edges of the powder spectra.

be ascribed to general ionic strength effects, since no spectral changes are apparent in control studies using NaCl. Different patterns of spectral behaviour are observed at all of the head group segments studied. Binding of $\text{La}(\text{III})$ or $\text{Fe}(\text{CN})_6^{3-}$ has little effect on the shape of the ^{31}P NMR spectra. But on addition of $\text{Eu}(\text{III})$ a large chemical shift is observed which is accompanied by an apparent reversal in the sign of the chemical shift anisotropy. The effects of ion binding on the quadrupole splittings of the $^1\text{NCH}_2\text{CD}_2$ and $^1\text{NCD}_2\text{CH}_2$ segments (the α and β positions, respectively) tend to be generally in the same direction, but the magnitude of the observed changes is ion-specific. The observed quadrupole splitting at the α position is decreased, whereas that at the β position is increased on ion binding. Little change is apparent at the $(\text{CD}_3)_3\text{N}^+$ segment (data not shown). The data are summarised in Table 1.

The mole ratios of ion to phospholipid used in this work are higher than those used by Hauser *et al.*³, however, concentration dependence studies reveal significant effects at ion : phospholipid mole ratios as low as 1 : 10. In the case of $\text{Eu}(\text{III})$ binding, two components are seen in the ^{31}P NMR spectra in certain conditions—the predominant spectral component is shown in Fig. 1. It is also possible that there is more than one component on binding of $\text{La}(\text{III})$ or $\text{Fe}(\text{CN})_6^{3-}$, as the lack of a chemical shift in the ^{31}P NMR spectra upon binding of these ions would make it difficult to resolve components with similar chemical shift anisotropies.

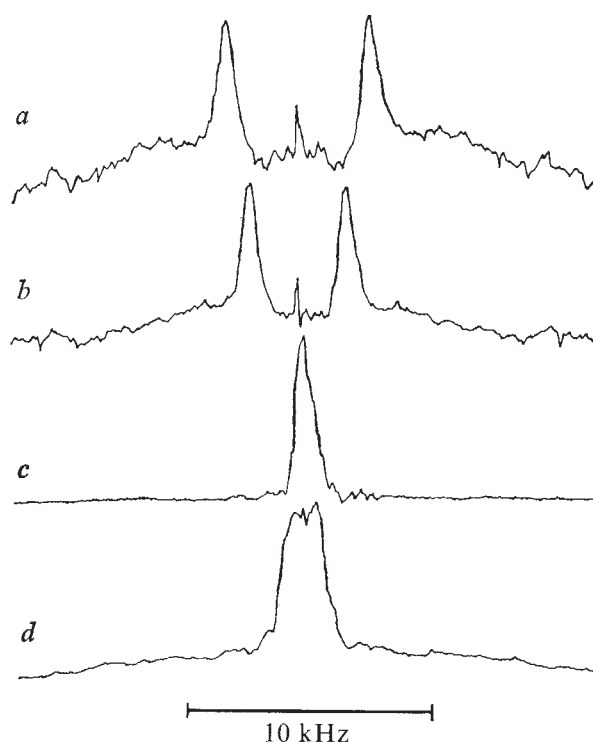


Fig. 2 Deuterium NMR spectra of unsonicated multibilayers of $^+NCH_2CD_2$ -DPPC complexed with trivalent ions at 59 °C. *a*, No ions; *b*, $Fe(CN)_6^{3-}$; *c*, $La(III)$; *d*, $Eu(III)$. Selectively deuterated DPPC was prepared as described in ref. 3. The spectra were recorded at 13.8 MHz with a fluorine lock; otherwise the experimental conditions are identical to those described in the legend to Fig. 1. Approximately 6,000–10,000 free induction decays were averaged to obtain the spectra. The absolute value of the residual quadrupole splitting ($\Delta\nu$) is given by the separation between the two largest peaks in the powder-type spectra. Note that the spectra are recorded at different gains.

Since DPPC dispersions complexed with trivalent ions undergo phase transitions at temperatures near the gel to liquid crystalline phase transition of pure DPPC (ref. 7 and H. U. Gally, personal communication), it is unlikely that the bilayer structure is disrupted on binding of any of the ions used here. The static electric field gradient tensor of the 2H labels primarily reflects the electronic structure of the bonds containing these atoms and is not affected by ion binding to the phosphocholine groups. The observed changes in the residual quadrupole splitting must, therefore, be due to changes in the head group motion and conformation. The exact nature and extent of these conformational changes can only be determined from a quantitative analysis of the 2H and ^{31}P NMR data, which will not be pursued here. The interpretation of the ^{31}P NMR data is not unequivocal, as it is possible that the contact shift due to

$Eu(III)$ binding⁵ may be different for the different components of the chemical shielding tensor. In principle, this could account for the different ^{31}P NMR spectral changes associated with $Eu(III)$ and $La(III)$ binding and would be consistent with the similar 2H NMR spectral changes observed on binding of these ions. The binding of $Eu(III)$ and $La(III)$ to phospholipids is believed to be very similar⁵. An alternative explanation, however, is that the contact shift is similar or identical for all the principal components of the chemical shielding tensor, in which case the ^{31}P NMR spectral changes upon $Eu(III)$ binding would reflect changes in molecular motion at the phosphate segment. Further work, for example, determination of the ^{31}P static chemical shift tensor of DPPC complexed with shift reagents, is needed before a firm conclusion can be reached.

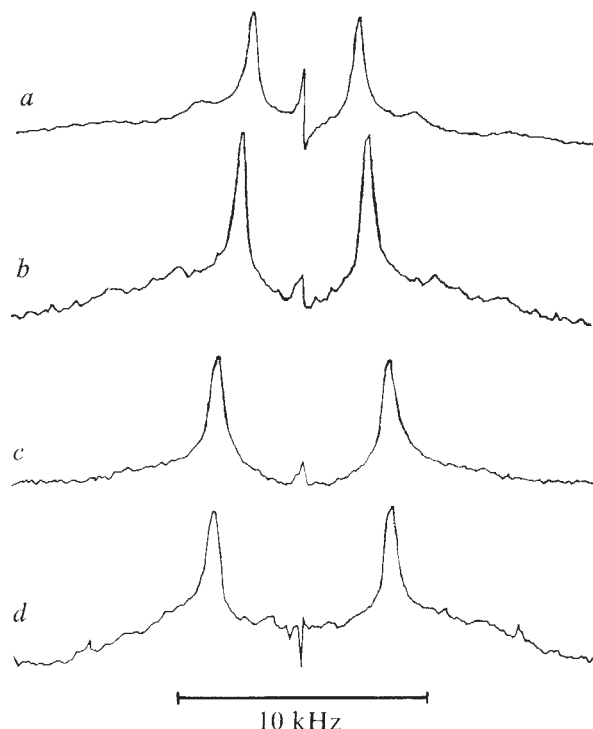


Fig. 3 Deuterium NMR spectra of unsonicated multibilayers of $^+NCD_2CH_2$ -DPPC complexed with trivalent ions at 59 °C. *a*, No ions; *b*, $Fe(CN)_6^{3-}$; *c*, $La(III)$; *d*, $Eu(III)$. Experimental conditions as in Figs 1 and 2.

The data presented here suggest that caution should be exercised in applying the results of NMR structural analysis using paramagnetic ions to phospholipid bilayers free of complexed ions. The observation of specific ion-induced changes is consistent with the idea that the phosphocholine group of DPPC is not in a fixed, rigid conformation^{3,4,8} and further suggests that structures deduced from data for binding of several ions^{3,6} may represent averages over several different conformations. These results are a direct demonstration of ion-induced changes in the head group conformation of phospholipids. Similar conclusions regarding lanthanide binding have been reached by Hauser *et al.* (manuscript in preparation). From a biological point of view, it will be interesting to see if divalent cations such as Ca^{2+} induce similar spectral changes and whether these changes can be quantitatively interpreted in terms of changes in head group conformation.

We thank Dr R. J. P. Williams for helpful comments. This work was supported by grant 3.008.76 from the Swiss National Science Foundation (J.S.) and a postdoctoral fellowship from the U.S. National Institute of General Medical Sciences (M.F.B.).

Table 1 Effect of trivalent ions on 2H quadrupole splittings ($\Delta\nu$) and ^{31}P chemical shift anisotropy ($\Delta\sigma$) of the phosphocholine group of DPPC

Segment	No. of ions added	$Eu(III)$ $\Delta\sigma$ (p.p.m.)	$La(III)$	$Fe(CN)_6^{3-}$
PO_4^-	-44	+44 $ \Delta\nu $ (kHz)	-54	-51
$^+NCH_2CD_2OP$	5.9	~ 1	~ 0	3.9
$^+NCD_2CH_2OP$	4.4	7.2	6.9	5.1

Ion solutions were prepared as described in ref. 5. $[DPPC]/[ion] = 2.7$ – 3.0 . $[H_2O] = 50 \pm 0.3\%$ by weight. Temperature, 59 °C.

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Replication of linear adenovirus DNA is not hairpin-primed

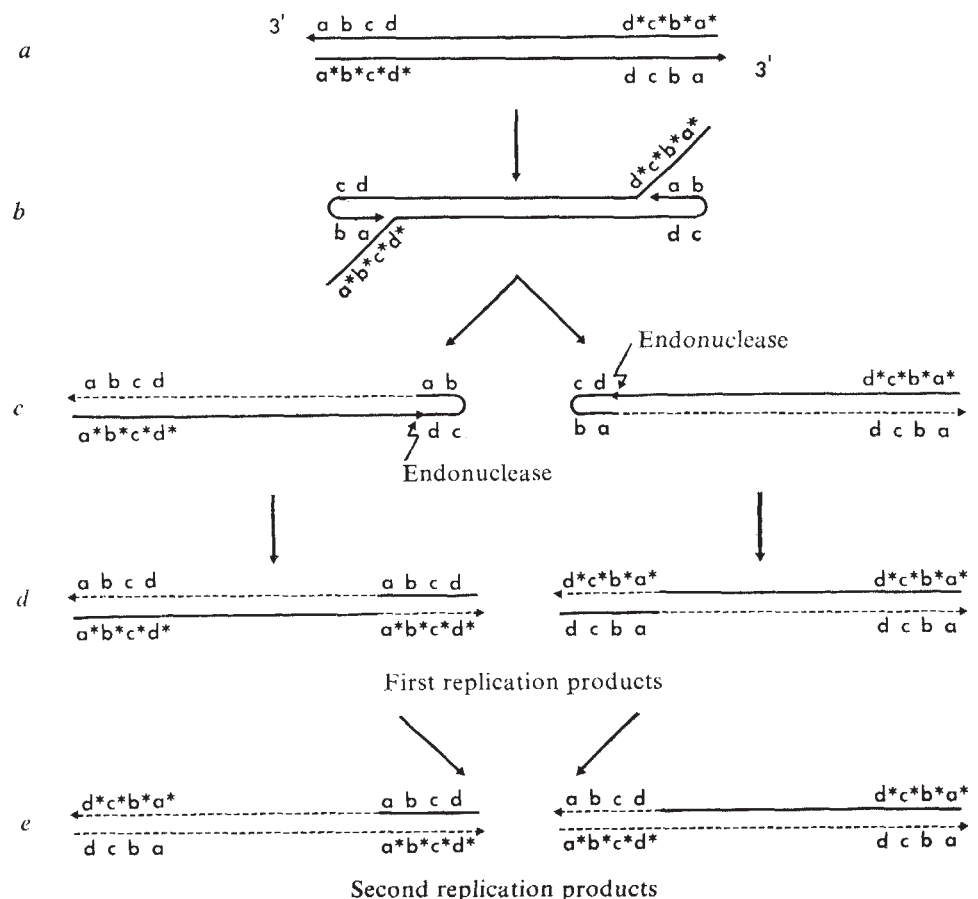
THE mechanism of replication of the 5' ends of linear DNA chromosomes remains unclear^{1,2}, as the known DNA polymerases, which act in the 5' to 3' direction, require a free 3'-OH as a primer for chain elongation. If a short ribonucleotide sequence primed DNA synthesis, its subsequent removal would leave no primer for gap fill synthesis at the 5' end. Most linear bacteriophage DNA molecules therefore have terminal repeats or single stranded complementary ends, which allow formation of circular or concatemeric replication intermediates³. Several interesting models have been proposed to explain replication of the 5' ends of eukaryote chromosomes and linear DNA molecules which cannot circularise. Cavalier-Smith³ proposed that chromosome ends can form self-complementary hairpin loops,

allowing the parental 3' end to act as a primer for DNA polymerase, followed by endonuclease action and repair synthesis. This model has been modified by Bateman⁴ and Tattersall and Ward⁵ have extended it to propose a rolling hairpin model for the replication of parvovirus and linear chromosomal DNA. There is now considerable evidence for hairpin priming of replication of the DNA of minute virus of mice (MVM) and adeno-associated virus (AAV)^{6–7}, and hairpin priming may well be a general mechanism for replication of linear DNA molecules that are unable to circularise or form concatemers^{3,5,7}. Roberts⁸ and Wu *et al.*⁹ have proposed a hairpin-primed model for replication of adenovirus DNA, which is summarised in Fig. 1. We present evidence here which is not consistent with a hairpin-primed model for replication of adenovirus DNA.

Human adenovirus DNA is a linear duplex and has ends with properties consistent with an inverted terminal repetition that is not palindromic¹⁰. J. R. Arrand and R. J. Roberts (manuscript in preparation) have sequenced the inverted terminal repetition in adenovirus type 2 and have shown it to be a unique, non-palindromic sequence of 102 base pairs (sequence a b c in Fig. 1) with an unpaired modified 5' terminal deoxycytidine. Covalently attached to each 5' end of the DNA is a 55,000 molecular weight (MW) protein¹¹, possibly linked to the DNA by the unpaired deoxycytidine residue. The DNA replicates by a strand displacement mechanism and the origins and termini of DNA replication are at the molecular ends of the chromosome^{12–14}.

One consequence of the hairpin-primed model (Fig. 1) is that with each round of replication, the terminal sequence must be inverted, resulting in four types of molecules differing in the order of the terminal sequences. As the adenovirus termini are not palindromic, the first round of replication products would have terminal repeats of two types and 50% of intracellular viral DNA should therefore anneal

Fig. 1 Model for self-primed initiation of adenovirus DNA replication by a hairpin mechanism. *a*, Virion parental DNA (solid lines) showing inverted terminal repetition (sequence a b c); *b*, hairpin formation at the 3' end of either strand (sequence a complementary to sequence d); *c*, daughter strand synthesis in the 5' to 3' direction using the 3'-OH from the parental strand as primer, and site-specific endonuclease introducing a ss nick near sequence d; *d*, strand displacement within hairpin and completion of terminal 3' ends; *e*, products of a second round of replication by the same mechanism. Model proposed by Roberts⁸ and Wu *et al.*⁹. The events shown could occur at the end of a round of replication (to complete the 5' ends of progeny strands) rather than at initiation as shown.



to form circles after digestion of the 3' ends with exonuclease III. We find this is not the case. Viral DNA was extracted from HEK cells infected with human adenovirus type 5 (Ad5). After digestion of 0.1%, 0.75%, 1.3% and 1.6% of the double-stranded molecules in one experiment and 0.1%, 0.7%, 1.1% and 1.5% in a second experiment, the intracellular viral DNA was annealed. Circular molecules that sedimented faster than linear Ad5 DNA were not observed (Fig. 2). Samples from one experiment were also examined in the electron microscope and no circular molecules were observed. In a control experiment with T7 DNA¹³, using similar digestion and annealing conditions, circle formation was observed in DNA digested to 0.9% of the molecule (Fig. 2) and 89% of molecules were circular by electron microscopy, whereas T7 DNA failed to circularise when digested to only 0.15%. These results indicate that non-inverted terminal repetitions of the type shown in Fig. 1*d* do not occur within the terminal 260 bases of replicating Ad5 DNA, as has been reported previously for mature DNA isolated from virions^{15,16}. As the terminal sequence is not palindromic (J. R. Arrand and R. J. Roberts, in preparation), we conclude that inversion of the termini during replication, which is a logical consequence of the hairpin-primed model for non-palindromic termini (Fig. 1), does not occur during adenovirus DNA replication. It is interesting to note that there is evidence for a terminal palindromic base sequence in AAV DNA⁶, supporting other evidence for a hairpin-primed mechanism for AAV DNA replication.

The hairpin-priming model also requires covalent addition of progeny DNA to a hairpin on the parental strand (Fig. 1). Some or all of the pulse-labelled viral DNA should therefore sediment faster than virion DNA in alkali and should contain sequences that spontaneously renature on rapid transfer to conditions in which duplex DNA is stable. But, fast sedimenting pulse-labelled adenovirus DNA has not been observed in alkaline gradients^{2,10,17}, and we have been unable to detect spontaneously renaturing sequences in replicating adenovirus DNA by chromatography on hydroxylapatite (data not shown). This may be because the replication intermediates predicted by the model are highly transient, but such intermediates were observed during AAV DNA replication^{5,18}.

We tested a further prediction of the hairpin-primed model for adenovirus DNA replication which is independent of the exact nature of the terminal sequences and whether self-priming occurs at initiation or termination of synthesis. It can be seen from Fig. 1 that the model predicts a strand switch in which the original 3' terminus becomes the 5' end of progeny strand, to be replaced by newly replicated DNA at the 3' end of the parental strand. Thus all newly replicated molecules should have newly replicated DNA at both 3' ends of the double-stranded DNA. This was tested experimentally by pre-labelling Ad5 DNA with ³H-thymidine and then transferring to medium containing ³²P and 5-bromodeoxyuridine (BUdR) (Fig. 1). If the model is correct, molecules that had undergone one round of replication in BUdR, isolated as a peak of hybrid (LH) density in CsCl, should have all their 3' ends labelled with ³²P. But, 50% of the 3' ends of LH DNA should be labelled with ³H rather than ³²P if there is no terminal strand switch during replication. The nature of the label at the 3' ends of LH DNA isolated from infected cells by CsCl equilibrium density centrifugation and hydroxylapatite chromatography was examined by exonuclease III digestion. Figure 3 shows the release of ³H-thymidine acid-soluble counts plotted as a function of the release of ³²P acid-soluble counts by exonuclease III digestion. The points form a straight line through zero, without the delay in release of ³H predicted by the model. The 95% confidence limits for the intercept of the curve indicate that a strand switch of less than 16

bases cannot be excluded, but such a short switch is not consistent with terminal sequence data (J. R. Arrand and R. J. Roberts, in preparation). Figure 3 also shows the expected curve if a strand switch of 120 base pairs occurred (that is, the smallest possible hairpin from sequence data). In this case, the intercept would be 0.68% ³²P counts released and zero ³H counts released, but this is clearly not the observed result. The discrepancy between our result and that predicted by the model is unlikely to be an experimental artefact. Contamination of LH DNA with ³H-LL DNA was not detectable by recentrifugation. Recombination between replicating molecules was not detectable as ³²P radioactivity entering the LL (³H) peak. Continued incorporation of ³H after the shift to ³²P, BUdR medium was prevented by adding a 33-fold excess of BUdR over the original ³H-thymidine concentration and addition of 5-fluorodeoxyuridine. Nearly all molecules labelled in a 3-h period would be mature, without gaps at which exonuclease could act, and completed molecules were further selected by using only the centre of the peak of LH molecules, and centrifuging the sample a second time in CsCl.

We have presented evidence that hairpin priming does not

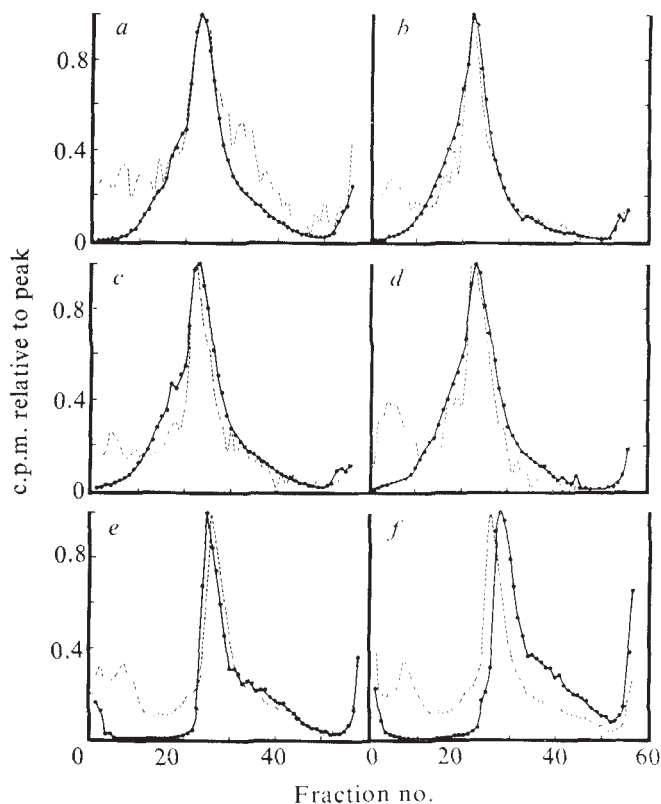


Fig. 2 Neutral sucrose gradient sedimentation of pulse-labelled Ad5 DNA and T7 DNA after exonuclease III digestion and annealing. Human (HEK) cells were infected with 5 plaque-forming units per cell Ad5 and labelled with ³H-thymidine from 16 to 17 h after infection. Viral DNA, isolated by a modification of the Hirt procedure²², neutral sucrose gradient centrifugation and hydroxylapatite chromatography, was digested for various times with *E. coli* exonuclease III, annealed for 30 min at 65 °C and slowly cooled to room temperature and centrifuged through a 5–20% neutral sucrose gradient (SW27, 18 h, 16,000 r.p.m. at 4 °C). ³²P labelled Ad5 DNA was added as marker (○); ³H-thymidine (●). Assuming Ad5 DNA contains approximately 35,000 base pairs, the extent of digestion from each 3' end would be 0.1% = 15 bases (a); 0.7% = 120 bases (b); 1.1% = 190 bases (c) and 1.5% = 260 bases (d). Lower panels: T7 DNA control digested to 0.15% (e) and 0.9% (f), annealed and centrifuged in similar conditions (●), with ³²P CELO virus DNA as marker (MW 29 × 10⁶)¹⁵. Sedimentation was left to right.

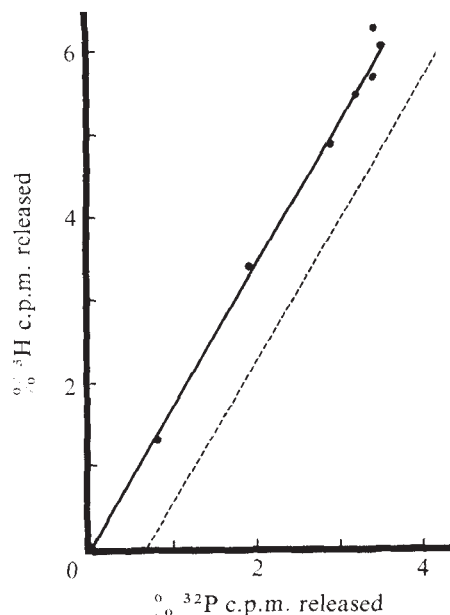


Fig. 3 Release of ^3H and ^{32}P acid soluble counts from LH DNA by exonuclease III digestion. Ad5 infected HEK cells were pre-labelled with ^3H -thymidine ($20 \mu\text{Ci ml}^{-1}$, $5 \times 10^{-7} \text{ M}$) from 6 to 16 h after infection and then washed and labelled with ^{32}P and $5 \mu\text{g ml}^{-1}$ BUdR, ($1.6 \times 10^{-5} \text{ M}$) in the presence of $0.5 \mu\text{g ml}^{-1}$ 5-fluorodeoxyuridine from 16 to 19 h after infection. Viral DNA was isolated by a modification of the Hirt procedure²², ribonuclease treated and centrifuged to equilibrium in a CsCl gradient (initial density 1.730 g ml^{-1}) in a Ti50 rotor at 33,000 r.p.m. for 64 h at 20°C . The centre of the LH DNA peak was collected and re-centrifuged in a similar equilibrium gradient, purified by hydroxylapatite chromatography and digested with *E. coli* exonuclease III. ●, % Release of ^3H counts plotted against percentage release of ^{32}P counts; dashed line, expected curve if the strand switch in Fig. 1 involves 120 base pairs (see text).

occur in adenovirus DNA replication. Wu *et al.*⁹ observed some type of secondary structure at the ends of adenovirus type 2 DNA in the electron microscope, but they could not conclude whether their data supported the hairpin structure shown in Fig. 1. There is no other published evidence for hairpin priming in adenovirus DNA replication, although there is considerable evidence for such a mechanism of replication at the 5' ends of other linear viral DNA chromosomes^{5-7, 18-21}. Although the absence of hairpin-priming in adenovirus DNA replication does not indicate the mechanism by which the 5' ends of progeny DNA are replicated, we have proposed an alternative model for adenovirus DNA replication in which the terminal 55,000 MW protein binds deoxycytidine and recognises the inverted terminal repetition, providing the free 3'-OH to prime DNA synthesis¹¹. This model is consistent with data presented above and all known data concerning adenovirus DNA replication, and suggests a function for both the 55,000 MW protein and terminal repetition. It will be interesting to determine whether replication of the ends of eukaryote chromosomes is primed by hairpin loops as originally proposed by Cavalier-Smith³, by a protein-bound nucleotide as we have proposed for adenovirus¹¹, or by some other mechanism as yet unknown.

We thank Dr H. B. Younghusband for helpful discussions and electron microscopy, Dr I. R. Lehman for *E. coli* exonuclease III and Miss A. Tandy for technical assistance.

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Received 13 July; accepted 12 August 1977.

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Benzo[a]pyrene diol epoxide covalently binds to deoxyguanosine and deoxyadenosine in DNA

CHEMICAL carcinogens, for example, benzo[a]pyrene (BaP), are metabolised to reactive intermediates which bind covalently to cellular macromolecules¹⁻³ and the extent of binding seems to correlate with the carcinogenic potency of the hydrocarbons¹. Through various mutagenicity, metabolism and binding studies, the intermediate which undergoes formation of a stable covalent complex with DNA has been identified as 7 β ,8 α -dihydroxy-9 α ,10 α -epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BaP diol epoxide)⁴⁻⁹. The structure of a covalent adduct formed between this hydrocarbon and poly(G) has also been established⁸⁻⁹. We report here the identity of several adducts obtained by reacting ($\pm$)BaP diol epoxide with tritium-labelled DNA. Four differently labelled lots of DNA were synthesised *in vitro* with DNA polymerase I by incorporating in each case three unlabelled and one tritium labelled nucleoside triphosphate. Through the use of this unambiguous labelling technique we have demonstrated that activated BaP forms two adducts with deoxyguanosine, two with deoxyadenosine and possibly one with deoxycytidine, while reaction with deoxythymidine was not detected. This approach also allowed the relative percentage of each adduct to be calculated. The deoxyguanosine adducts predominated and constituted 92% of the total stable covalent adducts formed.

($\pm$)BaP diol epoxide was synthesised as previously described^{10,11}. Covalent binding of adducts to calf thymus DNA was carried out at neutral pH by enzymatic activation of ^3H -BaP or reaction of unlabelled or ^{14}C -($\pm$)BaP diol epoxide directly with the nucleic acid. The phosphodiester backbone of the DNA was enzymatically hydrolysed and the products were chromatographed on Sephadex LH-20. Hydrophobic material eluting from the column was concentrated and further analysed by high pressure liquid chromatography (HPLC).

Figure 1a shows a HPLC profile of hydrolysis products resulting from a co-injection of unlabelled DNA reacted with (1) ^3H -BaP plus microsomes and (2) unlabelled ($\pm$)BaP diol epoxide. Chromatography of DNA-BaP diol epoxide adducts alone results in a fluorescence trace identical with that of Fig. 1a. But, chromatography of the microsome catalysed adducts alone results in detection of

peaks 1, 4, 5 and 7 only. Therefore, the reaction between ($\pm$)BaP diol epoxide and DNA yields three more adducts (peaks 2, 3 and 6) than BaP plus microsomes and DNA.

The bases to which ($\pm$)BaP diol epoxide binds were identified by reacting ^{14}C -($\pm$)BaP diol epoxide with each of four samples of ^3H -labelled DNA. The DNA samples were labelled unambiguously with ^3H in either deoxyguanosine,

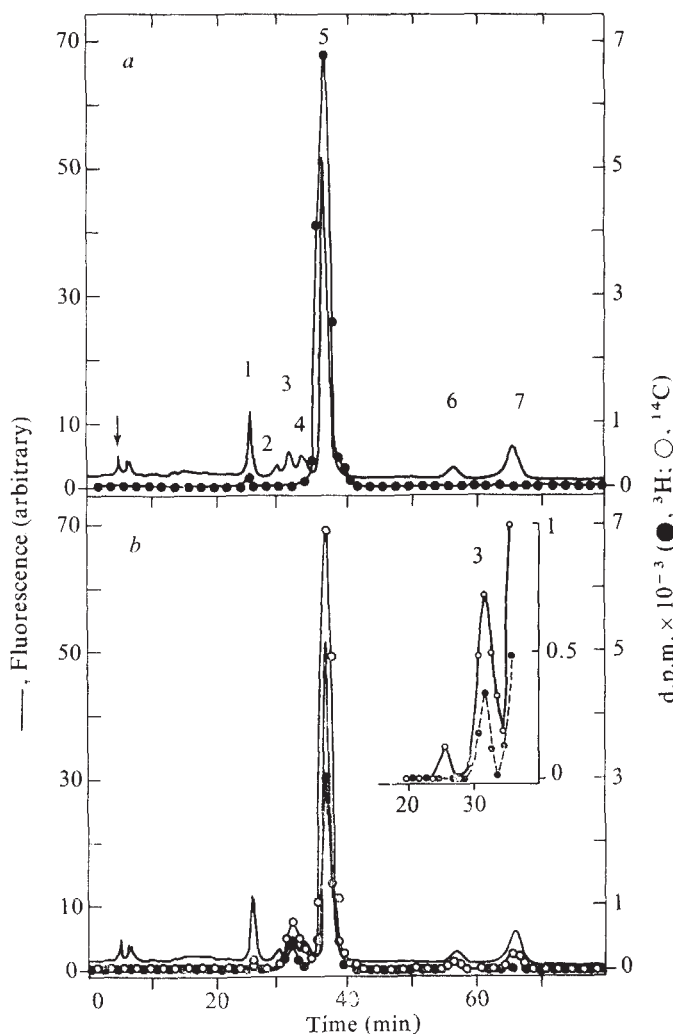


Fig. 1 HPLC profiles of BaP and ($\pm$)BaP diol epoxide-DNA adducts were carried out on a Varian model 8500 fitted with two columns (Waters μ Bondapak C_{18} , 4 mm $\times$ 30 cm) in series and eluted with 50% methanol water. BaP covalent adducts were isolated after reaction of [G- ^3H]BaP with DNA in the presence of NADPH and rat liver microsomes from animals pre-treated with 3-methylcholanthrene. Alternatively, unlabelled or ^{14}C -($\pm$)BaP diol epoxide was reacted directly with unlabelled or ^3H -labelled DNA in the same conditions as the enzyme activation of the hydrocarbon. The unlabelled or ^{14}C -($\pm$)BaP diol epoxide was used at the same concentration as the ^3H -BaP. The ^3H -labelled DNA's were enzymatically made by incorporation of three unlabelled and one ^3H -labelled nucleoside triphosphate with DNA polymerase from *Escherichia coli*. Calf thymus DNA, untreated, was used as template. The labelled nucleoside triphosphate was used undiluted and this procedure resulted in DNA with a specific activity of 14 $\mu\text{Ci mg}^{-1}$. Four DNA samples were synthesised with ^3H located in either (1) deoxyguanosine, (2) deoxyadenosine, (3) deoxycytidine or (4) deoxythymidine. After reaction of the hydrocarbon with DNA any protein was removed by phenol extraction. The DNA was precipitated with ethanol and the precipitate heated to remove intercalated hydrocarbon. The DNA was enzymatically hydrolysed and hydrophobic material isolated by Sephadex LH-20 chromatography. The HPLC profiles represent adducts obtained from LH-20 columns. *a*, Co-injections of ^3H -BaP plus microsomes reacted with unlabelled DNA and unlabelled ($\pm$)BaP diol epoxide reacted with unlabelled DNA and *b*, ^{14}C -($\pm$)BaP diol epoxide reacted with ^3H -deoxyguanosine-labelled DNA.

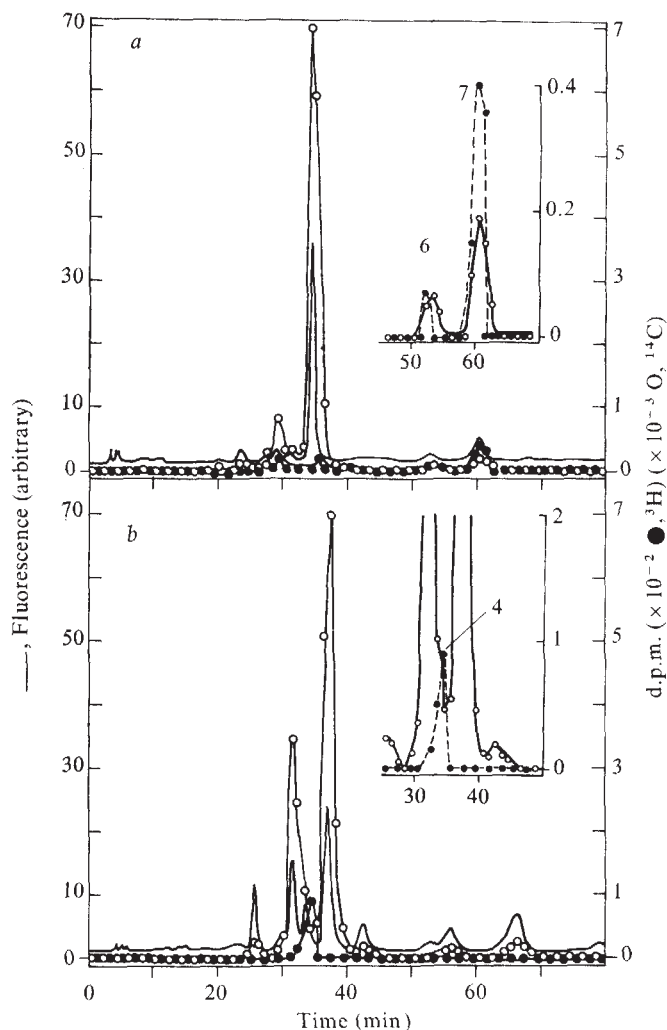


Fig. 2 HPLC profile of LH-20 samples isolated as described in Fig. 1 after reaction of ^{14}C -($\pm$)BaP diol epoxide with *a*, ^3H -deoxyadenosine labelled DNA and *b*, ^3H -deoxycytidine labelled DNA.

deoxyadenosine, deoxycytidine or deoxythymidine. The four samples of labelled DNA were prepared by incorporating three unlabelled triphosphates and one ^3H -labelled triphosphate into calf thymus DNA by *Escherichia coli* DNA polymerase I. The reaction conditions were essentially as previously described¹², except that the DNA template was untreated and the tritiated nucleoside triphosphate was not diluted with cold material. The labelled DNA was ethanol-precipitated, dialysed, reacted with ^{14}C -($\pm$)BaP diol epoxide and the covalent adducts were isolated after hydrolysis and Sephadex LH-20 chromatography. The results of three of the four double label experiments are presented in Figs 1*b*, 2*a* and 2*b*. HPLC of these samples resolved five ^{14}C -labelled adducts. Figure 1*b* represents reaction of ^{14}C -($\pm$)BaP diol epoxide with ^3H -deoxyguanosine labelled DNA. Peaks 3 and 5 contain both ^3H and ^{14}C and therefore represent hydrocarbon-deoxyguanosine adducts. From the specific activities of both the hydrocarbon and base a 1:1 ratio of the two components was calculated. Similar inspection of Fig. 2*a* and *b* identifies peaks 6 and 7 as deoxyadenosine adducts while peak 4 may be a deoxycytidine complex. Assignment of this adduct must remain tentative because of the lack of complete symmetry and coincidence of the ^3H and ^{14}C . Reaction of deoxythymidine was not observed despite our ability to detect approximately 1 adduct per 2.5×10^7 base pairs. The relative percentage of each adduct formed is shown in Table 1. Binding occurred to the extent of 92% deoxyguanosine, 5% deoxyadenosine, 3% deoxycytidine

Table 1 Relative distribution of ($\pm$) BaP diol epoxide adducts with the bases of DNA

Peak no.*	1†	2	3	4	5	6	7
Base	—	?	dG ₁	dC [‡]	dG ₂	dA ₁	dA ₂
Per cent total based on§							
³ H	—	—	10	2.4	86	0.1	1.4
¹⁴ C	—	—	10	3	82	1	4

*Peak nos refer to Fig. 1a.

†Peak 1 is probably a hydrolysis product of ($\pm$) BaP diol epoxide.

‡Assignment of this adduct is tentative.

§Computed from Fig. 1b.

and 0% deoxythymidine. These results are not in accord with those obtained by reaction of ($\pm$)BaP diol epoxide with model nucleic acids¹³, where the hydrocarbon reacted with poly(G), poly(A) and poly(C) to a similar extent. The reactions with model systems were carried out in 50% acetone which would have denatured the nucleic acids¹³, while the conditions used here were more favourable to maintaining helical conformation. The differences in reaction conditions may explain the relative lack of binding asymmetry found with the model systems.

Two adducts were obtained for both deoxyguanosine and deoxyadenosine and this could also be the case for deoxycytidine since peak 2 was not identified and could represent a second deoxycytidine adduct. The formation of two adducts with each base could have resulted either from the reaction with both stereoisomers of ($\pm$)BaP diol epoxide or alternatively a single isomer forming *cis* and *trans* addition products. Reaction of ($\pm$)BaP diol epoxide with poly(G) results in predominately *trans* addition of equal amounts of both stereoisomeric hydrocarbons⁶. We also analysed the adducts formed between ($\pm$)BaP diol epoxide and poly(G) by HPLC and circular dichroism spectroscopy. In confirmation of the results of Weinstein *et al.*⁵, we found that two major adducts were formed in about equal amounts and correspond to reaction of (+) and (−)BaP diol epoxide. In addition, two minor adducts were found which could represent (+) and (−)BaP diol epoxide reacting by *cis* addition of the guanine base.

Only one deoxyguanosine adduct was formed with the microsome system. This probably represents *trans* addition of only one enantiomer since enzymatic formation of BaP diol epoxide is stereoselective¹⁴. Two adducts were formed by reaction of ($\pm$)BaP diol epoxide with DNA and by analogy with the poly(G) system correspond to adduct formation with the (+) and (−) enantiomers of the hydrocarbon. In contrast to the poly(G) system where diastereomers are formed in approximately equal amounts, the DNA adducts occur at 90% and 10% for deoxyguanosine and 80% and 20% for deoxyadenosine. The amounts of the two adducts with deoxyguanosine and deoxyadenosine tend to equalise on binding of the hydrocarbon to heat denatured DNA. Also, there is an increase in the overall proportion of deoxyadenosine adducts. These results suggest that the asymmetric binding of the two stereoisomers of ($\pm$)BaP diol epoxide is less with single-stranded DNA than with double-stranded DNA. The preferential reaction of one enantiomer with DNA may be related to the dissymmetry of the right-handed helix, while the increase in deoxyadenosine adducts may result from topological changes in DNA conformation. Thus, the rates, sites and amounts of products obtained by reacting activated BaP with nucleic acids are probably controlled to a large degree by the amount of helical form and the conformation of the polymer. Of the adducts which have not been characterised the identity of peak 2 remains unknown while peak 1 co-chromatographs by HPLC with a hydrolysis product of ($\pm$)BaP diol epoxide. The high-resolution mass spectrum of the main deoxyguanosine adduct (peak 5) is

consistent with a structure in which the 10-position of the hydrocarbon is attached to the N²-exocyclic amine of the base. This structure is analogous to that obtained by reacting ($\pm$)BaP diol epoxide with poly(G)⁶. Quantitatively the most important adduct is binding to the exocyclic amine of guanine. Since ($\pm$)BaP diol epoxide formed adducts only with those bases containing a free amino group it is possible that this is the preferred binding site of the hydrocarbon.

In addition to the results reported here two other studies have indicated that BaP activation occurs by formation of the diol epoxide^{4,6}. But, evidence for other, as yet uncharacterised, intermediates has been presented^{15,16}. The difference in these results and ours may lie in the reaction conditions used. We find that magnesium chloride at 1 and 5 mM inhibits microsomal mediated binding of BaP to DNA by 60% and 75%, respectively. Since magnesium chloride was used in these studies^{15,16} it might account for the sixfold lower binding which was obtained compared with our results. If magnesium chloride specifically affected BaP diol epoxide binding another type of adduct could accumulate which would otherwise be a minor component. Even if other binding adducts do occur the importance of studying BaP diol epoxide rests on the observation that it is the most biologically active metabolite of BaP yet described^{8,21,22}.

In addition to the base adducts reported here, ($\pm$)BaP diol epoxide catalyses DNA and RNA strand scission¹⁷. Despite the fact that 250–300 base adducts are formed for each strand scission¹⁷ the relative importance, and indeed physiological significance, of each of these processes are unknown. Ethyl nitrosourea forms alkyl phosphotriesters and strand scissions which are lethal¹⁸. Since ($\pm$)BaP diol epoxide undergoes similar reactions this could account for some of the cytotoxic properties of BaP¹⁹. Furthermore, the excision repair of adenine carcinogen adducts in a number of cases is known to occur at a greater rate than those of guanine²⁰. The N²-exocyclic amine adduct of deoxyguanosine-BaP diol epoxide may therefore have a relatively long biological half life. As cancer induction occurs over a long period of time this may be an important adduct in the biological transformation caused by BaP.

We thank A. L. Burlingame for the high resolution mass spectrum and NCI for the ¹⁴C-($\pm$)BaP diol epoxide. This work was supported in part by the US ERDA Division of Biological and Environmental Research and NCI, contract Y01-CP50203.

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Received 3 June; accepted 15 August 1977.

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matters arising

General relativistic incompressibility

COOPERSTOCK and Sarracino¹ have attempted to redefine the concept of incompressibility in general relativity. Their result, if correct, would lead to a higher allowable maximum redshift from the surface of a bound object than is usually said to be permitted². We point out here a serious physical difficulty associated with their work. In calculating the mass of an equilibrium configuration in general relativity, the local mass density (a scalar quantity) can always be related to the pressure through an equation of state established in an inertial reference frame, without regard to the local gravitational potential. Nonetheless, Cooperstock and Sarracino choose to define a constant proper mass density $\rho_{\text{proper}} \equiv \rho(r)/g_{rr}^{1/2} = \text{constant} = a$ (say). They then compute a stellar mass from the relation

$$M = \int_0^r \rho_{\text{proper}} 4\pi r^2 g_{rr}^{1/2} dr \quad (1)$$

where g_{rr} is the radial component of the metric tensor. This definition of the mass is correct, being simply the general relativistic expression with $\rho_{\text{proper}} g_{rr}^{1/2}$ in place of $\rho(r)$.

As $\rho(r)$ is already a scalar, however, ρ_{proper} has no well defined physical significance. The division of the integral for mass into a product of a 'proper' volume and 'proper' density is arbitrary and misleading. Cooperstock and Sarracino, in asserting that the global contribution of gravitation to the overall mass-energy of a star will affect the local stress tensor, have missed the point of the equivalence principle, the very foundation of general relativity. The validity of this proposition guarantees that ρ itself (not $\rho/g_{rr}^{1/2}$) is what one would measure when applying an 'ergometer' to a small piece of matter, whether it was inside a neutron star or in empty space. In principle, the source stress tensor $T_{\mu\nu}$ appearing on the right hand side of the field equations $G_{\mu\nu} = kT_{\mu\nu}$ should contain all sources of mass-energy except gravitation. In practice, the gravitational binding energy of two neutrons, whether 10^{28} or 10^{-13} cm apart is negligible. Therefore ρ is the physical quantity relevant for local dynamical effects in neutron stars.

Nonetheless, following the procedure of Tolman³, one can treat the relation $\rho(r) = ag_{rr}^{1/2}$ as a defining equation for the radial variation of $\rho(r)$ and then deduce the equa-

tion of state $p = p(\rho)$. In that case, the equation of hydrostatic equilibrium is still given by⁴

$$\frac{dp}{dr} = -\frac{GM(r)\rho(r)}{r^2} \left[1 + \frac{p(r)}{\rho(r)c^2} \right] \times \left[1 + \frac{4\pi r^3 p(r)}{M(r)c^2} \right] \left[1 - 2GM(r)/rc^2 \right]^{-1} \quad (2)$$

Substituting $ag_{rr}^{1/2}$ for $\rho(r)$ and noting that in a physically allowable object p must be positive, all the terms on the right hand side of equation (2) must be positive, so that the pressure decreases with radius, that is $dp/dr < 0$. Now the numerical results of Cooperstock and Sarracino¹ show that $p(r)$ decreases with increasing $g_{rr}^{1/2}$, that is

$$dp/d(g_{rr}^{1/2}) < 0$$

But since $\rho(r) = ag_{rr}^{1/2}$ must be a positive quantity, $dp/d\rho < 0$. This is an unstable and unphysical situation. In order to have microscopic stability⁴, one requires $dp/d\rho > 0$. Furthermore, dividing dp/dr by $dp/d\rho$, one finds $dp/dr > 0$. It is hard to imagine that a physically realistic star with ρ increasing outward can be made. Though the resulting configuration is in equilibrium since it is a solution to the general relativistic hydrostatic equilibrium equation, it is unstable. Therefore, the resulting star constructed from Cooperstock and Sarracino's definition of incompressibility in general relativity is physically unrealisable. It seems that the redshift limit $z = 2$ set by Bondi² remains the largest allowable surface redshift consistent with both general relativity and microscopic stability.

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² Bondi, H. *Proc. R. Soc. A* **282**, 303 (1964); *Lectures on General Relativity*, Brandeis Summer Institute in Theoretical Physics, 1964 (eds Deser, S. & Ford, K. W.) (Prentice-Hall, Englewood, 1965).

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⁴ Weinberg, S. *Gravitation and Cosmology* (Wiley, New York, 1972).

COOPERSTOCK AND SARRACINO REPLY—
For Brecher and Wasserman, ρ_{proper} would have "well defined physical significance" only if it were completely invariant. In earlier correspondence, we had directed them to the equivalence principle, noting that this

principle renders such invariance *a priori* unattainable. The equivalence principle implies that the gravitational field can be locally transformed away by free-fall. That is not the point. The point is that with respect to a frame at rest relative to a spherically-symmetric body, ρ_{proper} certainly is well defined. It assumes the form $\rho g_{rr}^{-1/2}$ in Schwarzschild coordinates. This form has been justified by Misner and Sharp^{1,2} from dynamical considerations. We have justified the form from static considerations, and the extension has been made to charged fluid spheres (F.I.C. and R.S.S., in preparation, also, F.I.C. and V. de la Cruz, in preparation). In another coordinate system, say isotropic coordinates, it would not assume this form, but rather be determined by the integrand of the energy integral of the body over proper volume

$$M(r) = \int_0^r \rho_{\text{proper}} dV_{\text{proper}}$$

All energy, including gravitational energy, contributes to the total mass of the body and hence ρ_{proper} , which includes all energy, is the physically relevant quantity in general relativity. The failure to recognise its central role has been perpetuated by Newtonian conditioning.

On the one hand, Brecher and Wasserman assert that the validity of the equivalence principle guarantees that ρ and not ρ_{proper} is what their "ergometer" will measure. On the other hand, they then say that the gravitational binding energy of two neutrons, even 10^{-13} cm apart, is very small at any rate. If the energy is not there in principle, why worry about it in practice?

We feel that Brecher and Wasserman miss the point again. Certainly the gravitational binding for two neutrons is negligible. But, we are not concerned here about two neutrons nor indeed, necessarily about neutrons. We are concerned about conditions where large amounts of matter are being compressed towards their limit and gravitational energy is very significant indeed. This significance is made quite evident in the distinction between the bodies which satisfy the $\rho = \text{constant}$ and $\rho_{\text{proper}} = \text{constant}$ equations of state. We are not "asserting that the global contribution of gravitation to the overall mass-energy of a star will affect the local stress tensor ($T_{\mu\nu}$) . . .". We are asserting that the local contribution of gravitational energy, in addition to $T_{\mu\nu}$, determines the physically relevant proper total energy density.

Without entering the controversy regard-

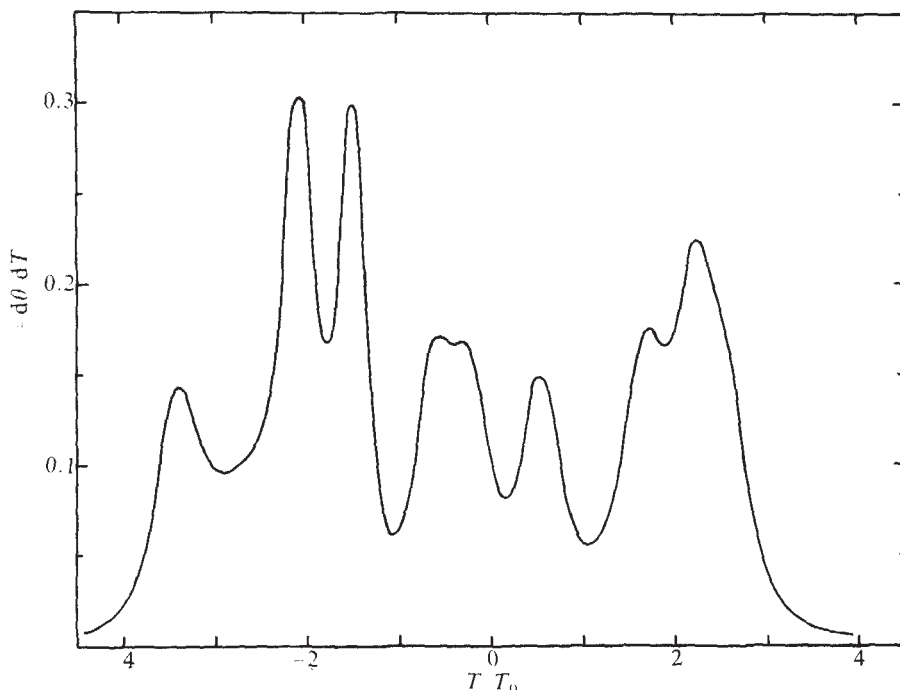


Fig. 1 Calculated differential melting curve for the block-random DNA sequence containing 4,002 base pairs. It consists of three blocks 1,334 base pairs long with GC contents of 0.43, 0.50 and 0.57. The sequences within each of these blocks were obtained using a random digits generator. The calculations were performed by the method of Poland⁸ for a standard set of the DNA thermodynamic parameters (see for example ref. 7). $\sigma = 5 \times 10^{-5}$; $T_{AT} = 340$ K; $T_{GT} = 380$ K; $U_{AT} = 8$ kcal mol⁻¹; loop-weighting exponent $\alpha = 1.5$. The negative value of the derivative of the degree of helicity θ with respect to temperature is plotted as ordinate and the difference between the temperature and the value $T_0 = 360$ K being an abscissa.

ing superluminal sound, we can certainly state that $dp/d\rho_{\text{proper}}$ is non-negative. One does not have to pursue the analysis of Brecher and Wasserman to determine that $dp/dr > 0$ for the $\rho_{\text{proper}} = \text{constant}$ equation of state. This is obvious from Buchdahl's proof. The point is that ρ_{proper} does not increase with r . To paraphrase Brecher and Wasserman, we would find it hard to imagine that a physically realistic star with ρ_{proper} increasing outward can be made. Clearly our equation of state was chosen as the limiting case which fulfills this requirement.

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The nature of the fine structure of DNA melting curves

SEVERAL reports¹⁻⁶ have suggested that the differential melting curves of DNA from various bacteriophages (including λ , T7, S_D, PM2 and fd) have a clear-cut fine structure. This fine structure is seen as a series of sharp peaks

with half-width of about 0.3° . Experimental results obtained in the various laboratories correlate well and thus one can suggest that fine structure of the differential melting curves is a general feature of all DNA's containing several tens of thousands or less of base pairs. It is clear also that a particular profile of the differential melting curve reflects a particular sequence of base pairs. Wada *et al.* have gone further and concluded that the fine structure is evidence of the existence in DNA of homostability regions characterised by highly homogeneous sequences largely different from random ones. They came to this conclusion because of their failure to obtain theoretically differential melting curves resembling experimental curves for the case of a random sequence of base pairs (see refs 2 and 4).

In contrast to the findings of Wada *et al.* we have shown⁵ that the existing helix-coil transition theory predicts the effect of fine structure in the differential melting curves for purely random sequences. The theory explains all main features of the observed effect including the width of the individual peaks, the dependence of the effect on number of base-pairs in the genome, on the state (closed or open) of circular DNA and so on (for details see ref. 5).

It has long been known that in many instances DNA consists of blocks

which differ from one another in their GC content, but inside such blocks the distribution of base pairs has been thought to be quasirandom (see for example ref. 7). λ DNA presents a classic example of such block sequences. It is clear that DNA with a block sequence will melt within broader temperature interval and simply because of this it will exhibit much greater fine structure than DNA of the same length but of random sequence (that is, consisting of a single block). In fact, in random DNA the different peaks overlap each other and smooth off the fine structure. Experimentally this tendency is shown dramatically when quasirandom T7 DNA is compared with the block λ DNA which is virtually as long but has a differential melting curve with very much more distinct fine structure.

An even more impressive example of a differential melting curve with very distinct peaks was presented by Wada *et al.*⁶ They measured the differential melting curve for the replicative form of fd DNA. This DNA consists of only about 6,000 base pairs but its differential melting curve contains as many as seven well resolved peaks.

To simulate something similar to this we have generated a sequence containing 4,002 base pairs divided in three blocks each consisting of 1,334 base pairs with the GC contents of 0.57; 0.50 and 0.43. These values were chosen so that the resultant melting curve for our artificial sequence should be as wide as the experimental curve for fd DNA. Within each block a sequence was obtained with the aid of a generator of random digits. The theoretical differential melting profile for such sequence is shown in Fig. 1. This profile consists of at least six well resolved peaks and bears a strikingly close resemblance to the experimental profile presented by Wada *et al.*⁶

Thus we conclude that the fine structure of differential melting curves can be explained quite plausibly by the theory of helix-coil transition under the assumption that only quasirandom or block-quasirandom sequences exist. So the observed sharp peaks on the differential melting curves can not be considered as evidence for existing homostability regions, that is, sufficiently long sequences consisting of repeating short segments which contain a fixed number of AT and GC pairs as Wada *et al.*⁶ have claimed.

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Crustal and *in situ* origin of Norwegian eclogites

KROGH¹ in a study of Norwegian eclogites concludes that all these eclogites can represent crustal rocks metamorphosed *in situ* during subduction to depths of 60–85 km. To show that eclogites are metamorphosed *in situ* demands that their conditions of formation are replicated in the adjacent amphibolite, or occasionally granulite facies, gneisses¹. In the western parts of Krogh's area eclogite equilibration conditions are $T \sim 800^\circ\text{C}$, $P \sim 23$ kbar¹. There are, however, no gneissic parageneses compatible with such conditions in which plagioclase is unstable². Thus applying geobarometric techniques^{3,4} to a pegmatite, said to be genetically related to adjacent eclogite⁵, maximum pressures are 12 kbar (ref. 3) or 13.2 kbar (ref. 4) for an assessed $T = 700^\circ\text{C}$. The occurrence of margarite in meta-anorthosites associated with eclogite⁶ gives maximum pressures of about 9 kbar⁷.

For Norwegian eclogites to be crustal demands that either their bulk chemical variations are controlled only by low- P fractionation, or that transitions from parent basalts and so on can be seen. I know of no unequivocal examples of the latter.

Krogh¹ does not seem to distinguish between the country-rock eclogites⁸ he describes and eclogites within ultramafic masses^{9,10}. He ignores critical evidence from the latter association that clinopyroxene, orthopyroxene and garnet show exsolution relationships^{9,10} which suggest high T – P conditions (1,100–1,600 $^\circ\text{C}$, 20–40 kbar) not compatible with crustal metamorphism or a subduction regime. He also ignores the description of orthopyroxene exsolving garnet from a country-rock eclogite¹¹ (recent work¹² suggests $T = 1,200$ – $1,370^\circ\text{C}$, $P = 30$ – 33 kbar).

Krogh demonstrates that there are regional variations in conditions of eclogite equilibration and that these conditions vary with time. He does not show how the eclogites reached this equilibrium. Exsolution data suggest that some eclogites at least,

formed from high- P liquids^{10,11} and are neither crustal nor *in situ*.

Paradoxically continent–continent collision processes¹ also seem a likely scenario for such rocks though dominated by deep level obduction¹² rather than subduction processes¹.

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KROGH REPLIES—From the first sentences and the conclusion of my article¹, it should be quite clear that I am dealing with eclogites within gneisses. Low- P (<10 kbar) fractionation may, in fact, be responsible for chemical variations within eclogites². Several lines of evidence also suggest that some of the eclogites may represent meta-sediments³. Transitions from parental low- P igneous to eclogitic parageneses are in fact, described from several localities (refs 4–7 and Bryhni & Wickström personal communication).

Gneisses from Hareidland contain plagioclase–clinopyroxene symplectites along with garnet + Ksp $\pm$ bio + rutile^{2,8}, thus indicating an originally eclogitic paragenesis in which plagioclase was unstable.

Analyses of co-existing garnet + clinopyroxene pairs from granulites at Måløy show that the $K_D^{\text{FeO/MgO}}$ values here are equal to those in adjacent eclogites (L. Malinconico, personal communication). This indicates similar metamorphic conditions in these two rock types.

Margarite in meta-anorthosite associated with eclogites is suggested by Lappin⁹ to be related to local amphibolitisation of the eclogites.

The pegmatite referred to¹¹ may well be from the field and petrographic descriptions⁸ post-eclogitic. Lappin's¹¹ limitations on the maximum pressure attained by the gneisses are thus related to later, post-eclogite events.

I have demonstrated the variation in metamorphic conditions with time by studying the chemical zoning of the minerals of the eclogites.

The inferred increase in P and T from, for example, 9.6 kbar, 505 $^\circ\text{C}$ to 17.6 kbar, 720 $^\circ\text{C}$ (ref. 1, Table 1) must be regarded as a rather marked variation. I further reported relict blueschist facies mineralogy in eclogites from Sunnfjord. In these samples, the cores of both garnet and amphibole have compositions typical for blueschist facies¹⁰, while the rim compositions are more typical for eclogites within gneisses^{10,12}. Furthermore, inclusions of low- P metamorphic mineral assemblages are fairly common in the garnets of eclogites from Nordfjord^{1,5,13} and Sunnfjord¹, indicating that prograde metamorphism has produced the eclogite mineralogy.

These variations all indicate that a large increase in P and T occurred with time at individual localities, as well as from east to west across the Gneiss Region.

With regard to the exsolution phenomena in pyroxenes^{14–16}, the exsolution history is still ambiguous. The existing chemical data are not satisfactory since the chemical zoning in the exsolved phases is not investigated. These data^{14–16}, however, still tend to give equilibrium conditions that fall within the regional P – T pattern defined for the whole area¹. A more extensive discussion of the crustal and mantle derivation of Norwegian eclogites will soon be available⁵.

The evidence of mineral zoning in individual samples, and the regional variation in equilibrium conditions strongly argues that the eclogites I have studied were formed by *in situ* prograde metamorphism of crustal rocks. This does not exclude the possibility that some of the components of this terrane, such as the ultrabasic masses, may have been introduced from the mantle^{5,11,16}.

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reviews

Wonderful contrivances

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Perpetual Motion: The History of an Obsession. By Arthur W. J. G. Ord-Hume. Pp. 235. (Allen and Unwin: London, 1977.) £.50.

THE subtitle of this book *The History of an Obsession* is significant in its place: the study is one of unrelieved failure (and a modicum of fraud); of inevitable failure when the products of obsessive fantasy are examined for sober fact. It is also apposite in another connection. In his Acknowledgements and Preface, and casually in asides throughout the text, the author gives a brief history of his own thralldom to his subject.

Mr Ord-Hume is a qualified mechanical engineer, with a successful career as a small-aircraft designer behind him, whose first encounter with the perpetual-motion saga was at his father's knee: "[My Father] used to tell me about it all when I was very small." Then, at university, his persistent importuning of one professor in particular at last brought the injunction never "to mention the subject again". In later life Mr Ord-Hume began a file of literature references to perpetual motion, as a by-product of research for other writings. The file grew and grew. Finally, a few years ago, the BBC's producer of *Horizon* planned a programme on perpetual motion, and he was asked to help. As he reports: "For several weeks I became thoroughly steeped in this subject, exploring in depth the references which I had filed away over the years . . . [the] material ended up in the form which you now have in your hands".

The form, alas, is not entirely prepossessing, there are footnotes with references to cited literature, there are four pages of bibliography and an index of more than six pages, indeed the trappings of a scholarly work, but the writing is far from scholarly. (On the author's behalf it should, however, be said that it was not his intention to write academically—rather to provide "a subjective history of the quest for the impossible".) Mr Ord-Hume should at least, however, have supervised his indexer. A single person referred to in different places in the text under slightly different guises (as Edward J.

Wood, rather than as Wood *simplificiter*, or as Edward Somerset, rather than as the Marquis of Worcester) is apt to be indexed under each guise, without cross-reference—and the author himself might well have considered the item "Ord-Hume, author, quoted" as supererogatory!

More serious, for whatever readership he was consciously writing, Mr Ord-Hume should have made sure that he got his elementary physics right. His version of Newton's first and third laws will pass muster, but his second law will not—"When a force acts upon a body in motion, the effect of this action is the same, in magnitude and direction, as if it acted on the body at rest." His version of the second law of thermodynamics reads: "heat cannot be increased without the expenditure of more work: it cannot run uphill".

There is much, then, to irritate the knowledgeable, or confuse the tyro, in this book, but there is also a wealth of solid information, and a gallery of illustrations (some ninety of them)

of the strange and wonderful contrivances from none of which, in the upshot useful work was obtained without drawing on an external source of energy (some, in fact, were never intended to do external work—the self-winding clocks, for example). The illustrations alone provide interest and instruction; it is convenient to have them in a slender (and not immoderately priced) volume. Apart from this aspect, however, the serious engineer will probably in the end find it necessary to seek out the two-volume work of Dircks (vol. 1, 1861; vol. 2, 1870) in the library, if he wishes to pursue the subject in depth.

Mr Ord-Hume may claim to have brought the story up-to-date by the inclusion of Strutt's radium clock, the Wilberforce pendulum and the breeder reactor as objects of discussion—but again, I fear, he has garbled the physics in the process. □

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Plant cell and tissue culture

Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture. Edited by J. Reinhart and Y. P. S. Bajaj. Pp. 803. (Springer: Berlin, Heidelberg and New York, 1977.) DM190; \$83.60.

PLANT CELL and tissue culture is developing into an active and exciting area of research and is permitting novel lines of investigation in the various plant sciences. *In vitro* techniques are being widely used: on the one hand they enable manipulations of microbial genetics, physiology and biochemistry to be accomplished with higher plant materials; whereas on the other hand, they prove a direct tool for the propagation of useful agricultural varieties. A major volume attempting a contemporary review of the field should reflect a fair measure of this excitement and potential. Indeed, there are some excellent and readable reviews in *Plant Cell, Tissue and Organ Culture* which capture interest and stimulate thinking. Several reviews of plant cell culture techniques

are particularly notable. These excellent gems are, however, set with uninspired efforts to make up this collection of thirty-five articles. Several of the reviews are either very poorly written or ramble from the declared topics.

The whole volume can be compared to that paragon of biological reviews, *The Bacteriophage Lambda*, published by Cold Spring Harbor Laboratories. One profound difference is immediately evident. Although the *Lambda* book seems to be a product of firm editorial control, this present book does not seem to have had such direction and, consequently, reads as if it were written by committee. Further, the *Lambda* book focuses on an organism, and thus, has a unifying theme, whereas this volume attempts to cover not only many organisms but also the entire complex phenomenology of plant cell, tissue and organ culture. Perhaps this is too much to ask of one book. We are left with the impression that the immediate details of the technology of *in vitro* plant culture overshadowed a coherent presentation of the underlying

biological principles. In a sense, the volume doesn't constitute a book (although bound as one) but a collection of reviews on *in vitro* plant culture, which could be the tissue culture chapters from books on specific crops, cytogenetics, development or other aspects of plant biology.

A closer look at a few of the chapters is instructive. Chapter 1 is called "Regeneration of Plants, Vegetative Propagation and Cloning". The authors of ten individual articles which comprise this chapter discuss many other aspects of plant cell and tissue culture besides these topics. A good number of them cover in elementary detail the very basics of plant cell culture, such as media composition and preparation of the explant; others attempt to cover all aspects of plant cell culture, including such items as haploids, mutagenesis, disease resistance and economics. The present chapter title is perhaps inappropriate and misleading, and could better be "Current Status of Cell and Tissue Culture with Regard to Selected Crops". This first chapter might have been organised around fundamental aspects of regeneration. What induces organogenesis and embryogenesis? What do we know and what do we need to know? What approaches are currently being taken to get answers to these questions? Then there could have been a final section on current applications of the *in vitro* techniques and further prospects. In organising the chapters by crops, the editors have permitted redundancy and inconsistency in the degree of detail.

Chapter 3, entitled "Cytology, Cytogenetics and Plant Breeding", doesn't

deal with plant breeding at all; that is, it doesn't give examples of breeding schemes or new varieties or individual plant breeders who utilise tissue culture techniques. There is also an inappropriate mixing of whole plant and tissue culture experiments. The reviews are typically a cataloguing of experiments, with little or no attempt to pull these findings together into a hypothesis (let alone, a theory!).

Chapter 4, "Protoplasts, Somatic Hybridisation and Genetic Engineering", contains very detailed reviews of the subjects it considers. In particular, each article tends to have full discussions of the experimental technique involved, although occasionally, at the expense of a critical evaluation of the implications of the data obtained. There is no mention of either recombinant DNA or crown gall—disappointing, since these are two very promising approaches to genetic engineering. Each article unfortunately contains small sections which repeat the material discussed as the main topic of another section.

Chapter 6, entitled "Cell Culture and Secondary Products", is very straightforward with three subsections. These include culture techniques, secondary products, and tissue culture and pharmacy. A nice addition to the extensive secondary products list in section two would have been the area of importance of these products. The author of section three was the only one to make a real effort to discuss the exciting range and importance of plant secondary products. This chapter contains a great deal of information which should be of value to

those contemplating, or presently involved in secondary product research, but may be a confusing compendium of chemicals to others. The excitement of the area—where it's been, where it is and where it's going—was not obvious.

Some positive suggestions may be in order. The editors might have written a reasonably in-depth introduction to each major section. This could have served the function of bringing loose ends together and pointing out unifying concepts. If the book is ever revised, as such a work might be, it could be offered in several smaller volumes, even in paperback. This would allow investigators to buy just those sections which are of most interest to them. In fact, each chapter could have been one paperback. The redundant sections would then have become useful, the price could have been cut considerably, and the material might have reached a wider market.

In spite of its deficits, *Plant Cell, Tissue and Organ Culture* will find its way into a great number of laboratories working with plant cell culture. It does contain a copious quantity of factual information and thus fills a need. It is a shame that the publishers have decided to fix the price so high, and that copyright laws restrict photocopying. A photocopy at one quarter the expense is attractive—but we don't want to introduce scientists into a life of crime!

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Atomic collision theory

Potential Scattering in Atomic Physics. by P. G. Burke. Pp. viii+138. (Plenum: New York and London, 1977). \$27.

ALTHOUGH many of the theoretical ideas underlying the scattering of electrons by atoms were developed in the 1930s, particularly by Sir Harrie Massey and his collaborators, it was not until the development of electronic computation in the early 1950s that really extensive calculations could be carried out to test these ideas. It was a happy chance that the start of Professor Burke's career (under Sir Harrie Massey at University College, London) coincided with this development, of which he took the greatest advantage. Now he is not only one of the leading authorities on the theory

of atomic collisions, but also on the complex techniques involved in the computation of cross-sections which are both of intrinsic interest and of practical importance in the theory of plasmas, of astrophysics and in parts of theoretical chemistry. With the author's reputation in mind, one turns to this little book with high hopes, which are not disappointed. Here is a marvellously lucid and concise account of the elementary parts of atomic collision theory admirably serving as an introduction to the subject.

The book starts with an account of scattering by short range spherically symmetrical potentials, which is then generalised to include long range Coulomb potentials and spin-dependent potentials. Following this basic material, elastic scattering of electrons by atoms or ions is discussed and it is shown how, at low energies, the effective range and quantum defect theories can be developed. This leads naturally into a discussion of resonance scattering

with a brief account of some of the analytical properties of the scattering matrix. Some of the most fruitful techniques for the many electron problem have been based on variational methods and these are discussed in some detail, together with the associated problem of finding bounds to the phase shifts. The book concludes with chapters on the Born series and its convergence, and on those approximations that have been developed for use at high energies, including the well known Glauber approximation.

Although much of this book could be read with profit by final year undergraduates, it will be of most use to those students who are starting research in atomic physics or quantum chemistry, for whom it will serve as an elegant introduction to the original literature and to the advanced treatises.

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Crab biology

The Biology of Crabs. By G. F. Warner. Pp. xii+202. (Paul Elek: London, 1977.) £6.95.

It is no easy task to write a book about crabs which will both appeal to and be bought by a wide audience. One approach is to produce a short cheap book which will enter the undergraduate market as a supplement to more general texts, a second is to aim for a more definitive work which will become a necessary standby for every research worker involved with the Brachyura. Dr Warner has made a brave attempt to bridge this gap.

First, I must indulge in that measure of nit-picking required of a reviewer to prove that the book has been read. On p81, *Inarchus* should read *Inachus*, and on p139 *Metopaulus* should be *Metopaulias*. More seriously, on p121, $a > 1$ for positive allometry and $a < 1$ for negative allometry, and not the converse as is stated. Generally, however, the book is accurate, very well written, and complemented by a set of extremely clear diagrams which are either original or redrawn.

My main criticisms deal with sins of omission rather than of commission—with the balance of the material presented in relation to the book as a whole. Naturally the criticisms will reflect my own interests and prejudices, just as the content of the book reflects those of the author.

In the first chapter on anatomy and basic function, the stomach is discussed and illustrated at length, whereas the entire reproductive system is dismissed in a half-page of text. In the third chapter compound eye theory is considered in some detail without being related specifically to the Brachyura. In the chapter on rhythms, tidal and diurnal cycles are covered fully, but annual cycles of reproduction and moulting are glossed over—by no means all temperate crabs have synchronised annual breeding periods.

The chapters on life styles and food and feeding are fascinating but all too short, whereas that on social behaviour is particularly detailed in comparison. In chapter 8 on life histories some mention of how growth can be determined by Hiatt's method or by suture tags would be worth including, and a simple diagram would profitably replace most of the long verbal description of the mechanism of autotomy. The penulti-

mate chapter attempts to bring some order to the confused state of brachyuran phylogeny. I must personally disagree strongly with Glaessner's viewpoint, and emphasise that the affinity of the Raninidae and Tymolinae are with the primitive Brachyura, and that of the Oxystomata with the other advanced Brachyura.

There is sufficient background information in this book to make it intelligible to the non-specialist, and because of this it is certainly a useful source of facts and ideas for the undergraduate who wishes to seek examples of biological theories in action. The ecological diversity, yet basically conservative morphology, of the Brachyura makes them an excellent medium for this purpose. The book is of more limited use to the research worker because of the background content and the restricted length. Few aspects are dealt with in real depth, and the number of references is small in this context. But it is the only book on crab biology available in English. It is also a readable book, and will certainly provide a good introduction to the group.

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Defining the virus

The Virus: A History of the Concept. By Sally Smith Hughes. Pp. xix+140. (Heinemann Educational: London; Science History Publications: New York, 1977.) £3.90; \$6.95.

FIFTEEN years ago, it was relatively easy to define a virus. Since then, it has become increasingly difficult. The 1960s were the hey-day of the virion—that is, the definitive, observable infective particle of a virus. It was a circumscribed, compact, convenient entity and, for any one virus, once it had been described, the virion was essentially characterised.

Eighty years ago the subject was shrouded in confusion because of the lack of knowledge. Paradoxically, it has become more difficult again now, because of a plethora of knowledge. For example, the distinction between viral and cellular DNA in the DNA tumour viruses has become increasingly blurred, and, so far as the bacterial viruses are concerned, the plasmids have obstructed the once clear picture of phages as bacterial viruses.

The concept of the virion as the essential and definable stage in the

biology of any virus was not easily won, and the slow climb from confusion with the bacteria to the molecular biology of the 1960s makes an interesting story. Needless to say, it was not a smooth one, and it went by fits and starts rather than by steady progress. The 1890s were a watershed, when the agents of tobacco mosaic and foot-and-mouth disease were characterised as novel and even bizarre.

Dr Hughes spent three years as a historian in an academic department of virology, and her book records well the period leading up to the 1890s, and in particular the words and thoughts of Ivanovski and Beijerinck, their relative contributions to the subject, and something of how they lived, moved and had their being. (Who would have dreamed that Beijerinck's father was a tobacco-nist?) The story after the turn of the century is told somewhat more briefly, and supplemented by useful tables and appendices. The whole book is well produced and remarkably low-priced. One of its attractions is that most of the story can be read in the course of an evening. Strongly recommended.

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BOOKS

ON PURE
AND APPLIED SCIENCE

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Ancient supernovae

The Historical Supernovae. By D. H. Clark and F. R. Stephenson. Pp.x+233. (Pergamon: Oxford and New York, 1977.) Hardback £8.50; paperback £3.95.

THE history of astronomy is a history of man struggling with new ideas and painstakingly trying to make accurate observations of celestial objects. Ideas were easier and their reporting is more widespread; flat earths, geocentric systems, perfectly smooth and spherical moons and planets, crop up in many of the contemporary books and manuscripts. Reports of observations are rarer and less easy to decipher. Some examples are Eratosthenes's estimation of the size of the Earth, the measurement of the Earth-Moon distance by Aristarchus of Samos, and the catalogue of 1,080 stars made by Hipparchus which not only gave the celestial latitude and longitude of each star but also divided them into six magnitudes according to their brightness.

Legend has it that one of the factors that motivated Hipparchus to perform this task was the appearance of a nova in Scorpio in 134 BC. Interestingly, two of the greatest astronomers of the past millenium, Tycho Brahe and Johannes Kepler, were also fascinated by the new stars which appeared in AD 1572 and 1604. Novae, supernovae and supernovae remnants also seem to have fascinated David Clark of the Mullard Space Science Laboratory, University College, London, and Richard Stephenson of the Institute of Lunar and Planetary Sciences, University of Newcastle upon Tyne, and motivated them to write this book; we should be thankful they did. Their task was one of the more difficult tasks in the study of the history of astronomy, the careful and painstaking perusal of historical records for references to new stars.

The book is divided into twelve chapters. The first sets the scene and convinces the reader as to the reasons why supernovae are so important. Not only are they one of the more spectacular of stellar events but also their remnants, the ejecta of the explosion, are amongst the most unusual and exciting astrophysical phenomena. Supernovae are thought to be responsible for pulsars, black holes, runaway stars, extended sources of radio emission, beautiful expanding nebulous objects, galactic X-ray sources and possibly gravitational radiation. About one supernova occurs in our galaxy every 120 years, the high degree of obscuration cutting down the number observed from Earth. Luckily, observa-

tions of supernovae and their remnants in other galaxies has increased our knowledge of these objects enormously.

The second chapter reviews the historical sources used—Far Eastern histories and diaries (remarkably detailed records from Chinese professional astronomers/astrologers going back to about 200 BC), Arabic astrological works, medieval European monastic chronicles and post-Renaissance European scientific writings. Chapter three considers three types of new stars recognised by Far Eastern observers—the quest stars, rayed stars and sweeping stars, the latter two being tail-less and tailed comets. It also contains a catalogue of pre-telescope novae and supernovae complete with durations and approximate celestial galactic coordinates. Chapter four discusses the characteristics of supernovae remnants and lists the rem-

nants within 10 kpc of the Sun. Chapters 5 to 11 present a detailed discussion of the new stars of AD 185, 386, 393, 1006, 1181, 1572 and 1604, the prime supernovae candidates. The book ends with the authors' thoughts on the evolution of supernova remnants and a review of the possible effects a nearby supernova would have on the Earth and its environment.

At times, this book tends to exude the musty, mothbally smell of a doctoral thesis, an impression that is not helped by the fact that the book is typed, all 232 camera-ready pages of it. It is still, however, a fascinating account of these enigmatic early observations and is well worth reading.

David W. Hughes

David W. Hughes is Lecturer in Astronomy and Physics at the University of Sheffield, UK.

X-ray structural analysis

Structure Determination by X-Ray Crystallography. By M. C. F. Ladd and R. A. Palmer. Pp. 393. (Plenum: New York and London, 1977.) \$35.40.

THE jacket description claims this book to be "an indispensable guide for advanced undergraduates and beginning postgraduates", as well as being of interest to the large number of workers using crystallographic structure determination as a research tool. It is the first reasonably comprehensive textbook on the subject to be published for several years, and its emphasis differs somewhat from its companions.

The introductory chapters on crystal geometry are extremely thorough; the detailed analysis of space groups and their symbols, in particular, should prevent much of the confusion which a newcomer to the field experiences on first meeting them. The following chapter describes preliminary examination of crystals, and is less satisfactory in that it gives considerable space to techniques not frequently used (optical properties, indexing of oscillation photographs) yet only three-and-half pages to Weissenberg and precession methods, one or both of which is used in virtually every structure determination. There is, furthermore, no mention of the determination of crystal system and unit cell dimensions from Weissenberg photographs.

Chapter 4 derives structure factor equations from first principles; as throughout the book, the derivations

are clearly expressed and attractively presented, and the worked examples (particular space groups, systematic absences) provide considerable illumination of the principles involved.

The detailed methods of X-ray structure analysis are introduced gently and relatively early via simple (but real) examples involving consideration only of special positions; it is doubtless encouraging to the beginner to see 'real' structure determinations before considering more complex topics such as Fourier series and Patterson functions. These are introduced in chapter 6, again clearly derived and with several detailed examples of the application of Patterson methods, leading naturally to a discussion of heavy atom methods and difference syntheses.

The major disappointment is the relegation of direct methods to a chapter headed "Some Further Topics". In recent years, many advances have been made in the theory and practice of direct methods, and yet no material is presented which is not in Stout and Jensen's *X-Ray Structure Determination* (Macmillan, 1968). Some mention should have been made of non-centrosymmetric direct methods and the MULTAN philosophy, at the very least.

One might disagree with the authors' choice of material and emphasis (which they defend in the Preface), but one cannot criticise the quality of presentation. It must be asked, however, whether this book represents any substantial advance on other (cheaper) books already available on the subject; regrettably, it does not.

P. G. Jones

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obituary

W. W. Bishop

DR. WALTER WILLIAM BISHOP, Head of the Department of Geology at Queen Mary College, University of London, died suddenly at his home on 19 February 1977, at the age of 45.

A native son of Birmingham, he graduated from the University there in 1952 with an honours degree in geography followed by a Certificate in Education. However, his interests turned towards geology and it was in this field that he received his Ph.D. degree at Birmingham in 1956. He went immediately to Africa as geologist with the Uganda Geological Survey and, through contacts with E. J. Wayland and L. S. B. Leakey, became involved with studies of Pleistocene deposits and the geomorphological evolution of the region.

By 1959 he had demonstrated the natural origin of the supposed "Kafuan" culture and provided evidence for morphological and tectonic control of the events that had led to the erection of a rigid climatic framework that now seemed insupportable. The fossil-bearing Miocene sediments also attracted his attention and his first African paper was a brief account of Miocene mammals from Napak. Further field work in Uganda and Kenya later led him to realise the important genetic relationships that existed between the Miocene fossil deposits and the volcanic cones throughout the region.

Thus, within three years of his arrival, was established the pattern of his many important contributions on the stratigraphy, geomorphology and volcanics of the Tertiary and Quaternary in East Africa, as well as his fruitful association with archaeologists, anthropologists and palaeontologists concerned with mammalian and hominoid evolution.

In 1959 he went to the University of Glasgow as Assistant Lecturer in Geology and Assistant Curator at the Hunterian Museum, but after three years returned to East Africa as Director of the Uganda Museum in Kampala and Lecturer in Geology at Makerere College, University of East Africa. These years were very productive and Bill Bishop soon became the recognised authority on the Tertiary and Quaternary of the region. At the Museum he was responsible for several new developments, such as the establishment of a Museum Education

Service. He organised a number of expeditions, including the Baker Centenary Expedition (1963) to study the geomorphological evolution of the Lake Albert rift valley, which led to a new picture of the stratigraphy and to important fossil collections from the Pliocene-Pleistocene Kaiso Beds.

The prospect of a teaching position tempted him back to London in 1965 as Lecturer in Geology at Bedford College, where Professor Basil King was already interested in research on the volcanics of the rift valley and directed the East Africa Geological Research Unit. This unit undertook responsibility for systematic geological mapping in the Lake Baringo basin, where important sedimentary deposits were found sandwiched within a long volcanic sequence; these beds provided valuable fossil material bridging a former gap between the middle Miocene and the late Pliocene. Radiometric dating was applied to the entire Miocene succession, thus establishing for the first time a good chronology for the faunas.

After moving to Queen Mary College in 1974, Professor Bishop continued his East African field work, partly in association with Richard Leakey, but also began work in Pakistan where a group from Yale University was re-examining the Miocene-Pliocene Siwalik sediments and their critical hominoid fossils. He was invited to become Director of the Peabody Museum at Yale and his death occurred only a few months before he was due to take up this appointment. Not long before he had been honoured by the award of the Prestwich Medal of the Geological Society of London in recognition of the outstanding contributions that had made him "a world leader in the very active field of African geology."

Always a co-operative, enthusiastic and helpful person, Bill Bishop was deeply involved in local, national and international bodies ranging from student societies to the Presidency of the INQUA Sub-Commission for Quaternary Stratigraphy of Africa. He served the Geological Society of London as a Council member, as its Secretary, and as Scientific Editor of the *Journal*, doing a great deal to build up both the Society and its publication. He was joint organiser of two symposia sponsored by the Wenner-Gren Foundation for Anthropological Research and edited the two books that have

become standard references for the time scale of hominoid evolution. In 1975 he organised a highly successful three day symposium on a similar theme for the Geological Society of London, as yet unpublished.

Dr Bishop was a lively and attractive speaker and was guest lecturer at many institutions. At meetings, his tact and insight smoothed over potential conflicts and his humour was infectious. His performances in amateur dramatics and his admirable voice in light opera will long be remembered, as well as the witty ditties with which he sometimes enlivened scientific meetings. His death is not only a great loss to science but also a severe personal loss to his many friends and colleagues throughout the world. His happy family life with his wife and two sons did much to sustain him in his busy existence and his love of natural history remains imprinted in them as his science does in his students and his colleagues.

H. B. S Cooke

J. A. V. Butler

JOHN ALFRED VALENTINE BUTLER, F.R.S., Emeritus Professor of Physical Chemistry at the University of London, died on 16th July after a short illness.

'J.A.V.', as his friends knew him, was born on St. Valentine's day in 1899. His early education was at Cheltenham Grammar School, and he took his first degree in chemistry at Birmingham University. After a short stay at the University College of Swansea he spent 12 years as a lecturer at the University of Edinburgh. Although he was a physical chemist, we know that during this period he came into contact with Edgar Stedman who, in the late thirties, was beginning his work on the composition of the cell nucleus. From the few comments we heard J.A.V. make about his relationship with Edgar Stedman at that time, we gathered that it was not always harmonious. Nevertheless an obvious interest in the chemistry of the cell nucleus was planted at that time, and was to emerge later.

In 1939 a Rockefeller fellowship took him to the U.S.A. where he also served as an executive officer in the British Commonwealth Scientific Office in Washington.

In 1946 he returned to England to

work at the Courtauld Institute of Biochemistry in London for three years, and in 1949 came to the Chester Beatty Research Institute to apply his physical chemical knowledge and techniques to the study of DNA. This began an interesting and exciting 17 years, during which he studied many aspects of the chemistry of the components of the cell nucleus.

It was in 1953 that I first met J.A.V., and being a very junior member of the technical staff at that time I had little to do with him in an official capacity. However, within six months he was telling me in considerable detail why my geraniums were dying, and the need for repotting them at frequent intervals. Although I believe he was basically a shy man, he was friendly with all members of his staff, and never hesitated to allow the students a day off for the Varsity Rugby match at Twickenham.

He will be remembered also, I am sure, by his friends at the Institute, for his absent-mindedness. I have seen him return from an academic board meeting with two hats, one on his head and one in his hand, hotly pursued by the rightful owner of the second hat. On another occasion, deep in conversation with a colleague in a café, he complained about not receiving any change, only to find it in his coffee when he stirred it!

The stories about J.A.V. are legion. His lack of pens and pencils, the gunfight he caused in Chicago by losing his brief-case, and his very, very long pauses on the telephone before he answered "Butler here". He was in all ways the epitome of the "absent-minded professor".

We will also remember well his method of crossing the Fulham Road. Head down, deep in thought, shoulders slightly bent, straight across, ignoring all traffic. A screech of car brakes was often the sign that J.A.V. was crossing the road.

He was however, clear and precise about his research. Although he began by applying his knowledge of physical chemistry to the structure of DNA, he soon became interested in the wider aspects of DNA control and his interests lead him into the fields of DNA synthesis, RNA synthesis and the functions of the chromosomal proteins. This was, of course, in the fifties when molecular biology was in its infancy, and most people chose to forget that DNA was associated with an equal amount of protein.

He felt that the best strategic approach to the cancer problem was to understand the fundamental aspects of gene control and he directed his department to this end. However, he was keenly aware that this was a long term

approach and once confided to me that he was sad we could do so little of immediate value.

Apart from his work within the Institute, his scientific interests were wide and varied. He edited *Progress in Biophysics and Molecular Biology* for over 25 years and his publications, apart from his research work, varied from a text book on chemical thermodynamics to a very successful series of books, written for the layman, on various aspects of cell and molecular biology.

He was appointed to the newly-established chair of Physical Chemistry at the Institute in 1952, and was elected a Fellow of the Royal Society in 1956.

After his retirement in 1966 he still took an active interest in the work and was often to be seen at scientific meetings. He frequently called in to see us and to hear about the latest work. His last visit was early this year, when true to form he left his brief-case in my room, fortunately remembering it before he left the building.

He was widely read, keenly interested in the arts and an amateur painter of some merit.

He married Margaret Lois Hope in 1929, and as all his friends who visited his home knew well, he had a very happy and contented domestic life. His three children, two sons and one daughter, all followed his lead and have made careers in various other scientific disciplines. *E. W. Johns*

F. H. Ludlam

F. H. LUDLAM, Professor of Meteorology and Head of the Atmospheric Physics Group at Imperial College, London, died on 3 June 1977 aged 57 years. Frank Ludlam studied clouds. His descriptions of the movement of air and water vapour and rain through clouds were so intense and graphic that one began to suspect that he had some supernatural power to actually identify himself with a cloud.

He saw so much. He would come into a lecture with perhaps 20 slides then spend all his time talking about the first, frequently drawing back from removing it because some new feature had just caught his attention.

Having made fundamental contributions to the microphysics of clouds during the 1940's while working at the Meteorological Office, he moved to Imperial College and away from the study of the details of processes towards the study of the organisation and dynamical structure of clouds. This philosophy culminated in his now

classical description of the motion of air in severe thunderstorms. Like all grand ideas this one could, in retrospect, have been seen coming for years, but Ludlam had it. He discarded what he called 'the haystack theories' of storms. These were illustrated by diagrams, and thinking, with arrows all over the place. He replaced it by a streamline theory showing where the air and water came from and where it went to. He took a certain delight in the fact that, working on a tiny budget, he made a very satisfying advance in a subject of great economic importance.

Subsequently his interest shifted progressively to phenomena of larger scale. For his inaugural lecture in 1966 he showed that weather systems could be seen in a new perspective by examining them relative to axes moving with the system: again almost identifying himself with the phenomenon. This was his way of using the abstract concepts of pattern movement as distinct from pattern development developed by his penetrating contemporary, Eady. But whereas Eady's concepts were based on distinction of mechanism and arose from mathematical elegance and clarity, Ludlam made them lead to pictorial and functional beauty.

No man showed better the false distinction between artist and scientist. To him good science made a pleasing picture. He delighted in the impish gesture. Few present at a lecture to the Royal Meteorological Society (on three-dimensional data analysis) will forget the impact made when he projected a slide of a Rodin nude (very female) in the middle of a masterly description of the techniques of synoptic analysis.

In his magnum opus entitled *Cloud Physics* to be published by the Pennsylvania State University Press, he summarises his views on atmospheric phenomena from a few microns in scale to 40,000 km. He was concerned with the notion that conventional physicists, wedded to the controlled experiment, did not understand the essence of meteorology as he saw it. What he did see was an immensely elaborate system with complex feedback. The essence was the feedback and the uncontrollability of the meteorological experiment. He was the privileged onlooker. He even took lightly some of his beautiful and fundamental work on cloud microphysics and said it did not really matter if it were wrong: the atmosphere would find some way of fulfilling a more over-riding purpose whatever the details of the processes.

Such an integrating philosophy is likely to be fundamental for the study of complex systems like the atmosphere and we are privileged to have had so talented an exponent of it.

John Green

what's new-spectrometers

In addition to the regular Newly on the Market section in *Nature*, special features on a particular type of laboratory equipment or instrumentation will be published in this and forthcoming issues.

The purpose of a special feature is not to give listings of every item in the range of the manufacturers mentioned but to take a variety of equipment of interest in that feature area and give an idea of what kinds of instruments are available and from whom. There is a Reader Enquiry Coupon facing the inside back page.

Anaspec introduce a completely new type of microspectrophotometer, which will record absorption, emission, scattered or fluorescence spectra of any object capable of being viewed in a microscope. This revolutionary system is a miniaturised double monochromator spectrophotometer, that can be attached to a standard trinocular microscope, such that the only limit to sample size is the actual microscope image magnifying. The system is manufactured in the U.S.A. by Nanometrics Inc. and consists of a number of separate modules which can be purchased separately, according to need or application. These modules include microscope, spectrophotometer, wave length programme, recorder and a micro computer for more complex analysis. Applications include almost every area of life sciences, microbiology, geology, forensic science, chemistry and electronics. For example, the system can measure the absorption spectrum of a single chromophore in a cell, or in forensic studies can plot a spectrum from a part of a single fibre. In addition the system is ideally suited to measuring semi-conductor film thickness in an area as small as 0.001 mm.

Although NanoSpec/10 is designed primarily for micro work, it also has application to large area analysis on the same specimen, *in situ*, where before-and-after differences over a wavelength range caused by chemical change, migration, photosynthesis, chromatography or other effects can be computed and recorded. This is not possible with conventional instruments. Any specimen which can fit onto the

microscope stage can be studied in this manner.

Circle No. 45 on Reader Enquiry Card **KeveX** present several new systems. The nuclear fuel analyser is a way of achieving rapid, non-destructive and automatic elemental analysis of reactor fuel elements with a new computerised X-ray energy spectrometer (XES) system. Two solid state silicon detectors are multiplexed to double the spectrometer's count rate capability. 100% element certification is achieved on the order of 10 s per nuclear fuel pellet. The system is completely automatic with the computer selecting and controlling: X-ray tube voltage and current, secondary targets and filters, specimen transport, acquire and analyse data and sample advance. Other applications include: effluent analysis, pollution analysis and accurate accountability of special nuclear materials. Little or no sample preparation is required. The system can be employed as a materials monitor in response to the challenge posed by nuclear proliferation. The Ultra-Trace has the



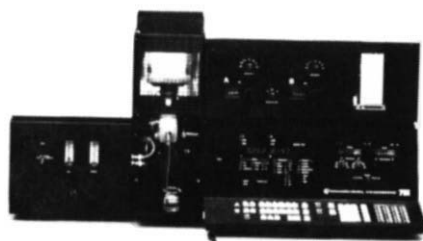
unique ability to analyse many ultra-trace elements simultaneously, non-destructively and rapidly. The minimum detection limits for elements of atomic number 23 through 92 are on the order of 50–100 pg. Minimum detection limits for elements of atomic number 9 through 22 are somewhat higher. Ultra-Trace uses new and advanced X-ray energy spectrometry

technology. This new product will provide process control chemists, the pharmaceutical industry and medical researchers with high speed, high dynamic range analytical capabilities. Quantitative element analysis of thin films and surface layers (e.g., oxides) are achieved with a new product and a new surface analysis technique. A new product is the KeveX Alpha-X, and the new surface analysis technique uses 5-MeV α particle induced X-ray emissions. Windowless detector-cryostats and radioisotope source-collimator subsystems are combined to yield heretofore unprecedented analytical performance. Rapid non-destructive quantitative analysis of elements of atomic number 6 (carbon) and up of surface layers are now possible using the KeveX Alpha-X. For example, carbon and oxide layers of less than 15 Å can be detected. Alpha-X spectrometers appear in two configurations: (1) integrated stand-alone systems and (2) attachment or accessory surface analysers. The accessory version of Alpha-X may be attached to existing Auger/ESCA type surface analysis chambers to yield complementary information. The stand-alone system is capable of both surface and bulk material analysis.

Circle No. 46 on Reader Enquiry Card

Instrumentation Laboratory (UK) Ltd introduces the IL 751 dual-channel double-beam atomic absorption spectrophotometer with built-in microcomputer. The new IL 751 Atomic Absorption Spectrophotometer is a dual-channel double-beam instrument which includes a microcomputer to make all the required computations. Each channel contains a full-size 330-mm monochromator, and both channels can operate with deuterium arc background correction. The two-channel system can produce great improvements in atomic absorption analysis, when used in either of its two main modes: (1) Doubled analytical speed. The IL 751 can analyse samples for two elements at one time. Conventional instruments can only determine one element at a time. (2) Greatly improved analytical accuracy and simplified sample preparation. In the internal standard mode, the absorbance of the element being determined is compared to that of an element having a known

concentration. This technique overcomes many matrix effects, such as sample viscosity or solution temperature, and minimises the effects of errors in pipetting or dilution. The IL 751 includes a microcomputer which performs all the calculations that are useful for atomic absorption data handling. Among these are linearisation of the analytical curves in both channels, using two, three, four, or five standards; readout in concentra-



tion in single-channel, dual-channel, or internal standard modes; peak height and peak area measurements; and provision of statistics including the mean, standard deviation, and RSD values. There are two special features: one is a keyboard-lock mode, which secures a program from disruption by an unskilled operator or curious passer-by. The other is a standby mode, in which the computer retains its calibration program even when the instrument is switched off. The IL 751 also contains a built-in alpha-numeric printer-sequencer, which identifies the results in addition to printing them out.

Circle No. 47 on Reader Enquiry Card

Techmation announce a new plasma emission spectrometer. A new single element plasma emission spectrometer, Spectraspan IV, will be available at a price normally associated with atomic absorption units. The highly versatile Spectraspan IV, developed by SMI, can perform quantitative and qualitative analyses of trace concentrations including refractories and some non-metals. It has a high sensitivity even in the presence of complex matrix solutions with solids contents as high as 20%. The high intensity excitation source is a d.c. argon plasma which operates at low power but produces temperatures as high as 10,000 K. As with the Spectraspan III Multi-element analyser, an integral microprocessor not only increases the efficiency of the system but allows routine checks of instrument performance. Plasma emission spectrometry has applications to a wide variety of environmental samples of unknown or varying composition: including trace elements in storm drain outfalls, sewage, sediments, soil, animal tissue, oil, plastics, sea water (including

measurements of mercury concentration as low as 0.15 p.p.b.), distilled water, brackish water, waste from plating baths, trace metal impurities in alloys and total phosphorus in storm drains.

Circle No. 48 on Reader Enquiry Card

Perkin-Elmer announces the first microcomputer-based UV-VIS-NIR spectrophotometer. Designed to meet the highest possible standards, the Model 340 offers three important benefits to the user. The first is ease of operation, the second is high performance enabling highly accurate photometric determinations and the third is versatility in sample handling, obtained through convenient instrumental operating conditions and well designed accessories. The heart of the Model 340 is a microprocessor which monitors all instrumental functions. It controls source and filter changes, the stepper motor monochromator drive, the integral X-Y recorder, zero and 100%T calibrations, response times, auto zero and automatic baseline correction, etc. Covering the range 190–260 nm with a resolution of 0.15 nm, the Model 340's double monochromator reduces stray light to 0.0002%T at 300 nm; the microprocessor enables a baseline flatness of ± 0.0014 in the UV/VIS region and 0.002A in the NIR region, to be achieved. A wide variety of sample handling accessories are available. The Model 340 can be equipped with long path cell holders, thermostatted cell holders and flow-cells. A complete line of integrating spheres enable precise measurements on solid and turbid samples over the entire wavelength range. A new addition to the 97 Series of IR Spectrophotometers, the Model 597, has been designed to meet the



Perkin-Elmer Model 373

growing need for instrumentation providing reliability, accuracy, versatility and simplicity of operation at a moderate cost. A high energy double beam optical null spectrophotometer, the Model 597 features an extended wavelength range of 4000 cm^{-1} to 200 cm^{-1} . Four scan speeds are offered,

three slit programmes and a time drive facility allowing transmission changes with time to be recorded on the instrument's own Flowchart recorder. It is compatible with Perkin-Elmer's new Infrared Multisampler which allows up to thirty samples to be run completely automatically. For analyses in the region 250 cm^{-1} to 200 cm^{-1} , where dry air or nitrogen purging is recommended, the sample compartment has been fitted with a sliding lid. The unique check gain control allows the gain to be accurately and reproducibly set, even when the lid is closed and a sample is in the beam.



Perkin-Elmer Model 597

The double beam, UV-Vis Spectrophotometer, the Model 556, is ideally suited for applications involving turbid samples, mixture analysis and many other biochemical and industrial applications. Two separate diffraction grating monochromators, with independent selection of wavelength, produce the sample and reference beams which pass along identical paths through the sample cell. To record the spectrum of a turbid sample where no reference solution exists, one monochromator can be fixed and the other set to scan. Alternatively, the two monochromators can be fixed a few nanometers apart and both scanned together to record the first derivative of a spectrum. A simple push button action converts to double beam operation with both sample and reference beams taken from one monochromator. Regular difference spectra can then be recorded with a performance level equal to many dedicated double-beam instruments. The instrument's XY recorder provides recording of the complete spectra on precalibrated chart paper, and allows step-wise expansion and concentration. Charts are conveniently stored on a pull-out roll; automatic pen lift and scan synchronisation are provided. Superimposed repetitive scanning is possible between pre-set limits using controls that read out directly in wavelength. Spectra can be recorded at eight scan speeds (up to $1,200\text{ mm min}^{-1}$) with automatic selection in reverse.

Circle No. 49 on Reader Enquiry Card

The Schoeffel Instrument Corporation introduces a new Universal High Sensitivity Spectrophotometer, HS 870, which accepts both microliter and milliliter type cuvettes to enable the researcher the option of using the instrument as a continuously variable wavelength monitor for HPLC or to do standard spectrophotometric analysis. This two-in-one concept saves the experimenter both money and valuable space. This HS 870 uses a true double-beam approach which assures a superior signal-to-noise ratio and maximum stability. The unit features a digital data display as well as three switchable modes of operation permitting measurement of transmission, absorbance, and concentration. An automatic gain control (over two orders of magnitude) and a zero suppression (total range greater than 2 A.U. or 120%T) are standard. A BCD output for computer interfacing is optionally available. The incorporated continuously variable monochromator with wide wavelength range (190–700 nm) enables the user to select the maximum absorbance of the compound of interest. This unit is modular in design with an emphasis on practicality.

Circle No. 50 on Reader Enquiry Card

Varian announce a new, moderately priced ultraviolet-visible spectrophotometer, featuring a double-pass monochromator and exceptional photometric linearity. The Varian Cary Model 219 UV-Visible Spectrophotometer has a resolution of less than 0.07 nm and a stray light characteristic of less than 0.002% at 220 nm. Photometric linearity is 0.0016 at 1.4 to 0.03 at 3.04. Ease of operation is enhanced by the instrument's wavelength coupled recorder, automatic 0%T setting, automatic source changing, automatic baseline correction and automatic repetitive scanning over the entire 185–875 nm wavelength range. Other features are a five-digit absorbance readout for high-precision measurements, expanded recorder ranges for instrument versatility and a large sample compartment preplanned for current or future accessory additions. Modes of operation include single beam for wavelength accuracy checks and double beam for normal absorptometric applications. Either auto-slit (constant noise) or auto gain (constant resolution) can be selected when operating in the double beam mode. A beam interchange control on the front panel electrically reverses the function of the sample and reference beams.

Circle No. 51 on Reader Enquiry Card

Philips present a variety of spectrometers and ancillary equipment. The PW 1600 is a simultaneous X-ray spectrometer fitted with a 12-position sample changer and fast printer. This versatile system—which permits the analysis of up to 28 elements in under a minute—is designed to meet the demand for high volume quality control analyses in the metal, mining, cement and similar industries. There is a new 3 kW X-ray generator—the PW 1730—together with vertical and horizontal goniometers, a tabletop X-ray generator, two Debye Scherrer cameras with advanced safety features, a radiation alarm monitor specially suitable for monitoring 'soft' X-rays and the latest generation of Philips X-ray tubes. The PW 1730, can be used for single tube and



Philips PW 1730 system

two tube sequential or simultaneous operation. High voltage is variable between 20 and 60 kV and tube current between 10 and 80 mA. Stability of kV and mA is 0.002% per 1% mains voltage variation and temperature drift is 0.002% per 1 °C ambient. Maximum output power is 3 kW. To save space and cost the X-ray programming and control electronics can be housed in the generator cabinet. Where additional modules are required—such as in highly automated systems—these can be housed in the optional PW 1735 Extension Console which also includes provision to mount a second X-ray diffraction tube tower. With the many options offered, the PW 1730 can be easily upgraded as needs change and larger systems are required. For example, a simultaneous control unit allows two X-ray diffraction tubes or one X-ray diffraction and one X-ray spectrometry tube to be used simultaneously. A high voltage switch enables two X-ray tubes to be used sequentially. These accessories

eliminate the need for a second generator in many applications—a very significant cost saving. High voltage, filament supplies, safety devices and cooling water are all automatically controlled for both tubes when either a high voltage switch or the simultaneous control unit is fitted. An additional overload protection unit is also available to protect tube(s) and generator during single or two tube sequential or simultaneous operation. The PV 8350 emission spectrometer is a direct reading vacuum spectrometer providing fast quantitative measurements at a cost that makes it a practical quality control tool for even relatively small steelworks and foundries. Typically it determines up to 20 elements simultaneously, including carbon, sulphur, phosphorus and boron. The EXAM 6 energy-dispersive X-ray fluorescence system is for simultaneous elemental analysis in such fields as metallurgy, ceramics, cement, food and forensic studies.

Circle No. 52 on Reader Enquiry Card

Glen Creston Instruments Ltd present the Spex Fluorolog Spectrofluorometer and the Ramalog 5M/6M Laser Raman Spectrometers. In addition to the standard emission and ratio emission measurement, it is possible to measure transmittance, absorbance and partial fluorescence efficiency without changing the configuration of the Fluorolog. In the ratio emission (or emittance) mode, excitation spectra are corrected automatically by the reference detector. Emission and higher order corrections are possible with the computerised counterpart of the instrument. This incorporates the necessary computer and software. A phosphorimetry attachment is available as well as many other accessories such as sample heater/cooler, variable temperature accessory and polarisation kit. The Spex Fluorolog and its computerised counterpart, the Fluorocomp, are new research systems and any type of luminescence can be measured by the Fluorolog. Its modularity enables change of the source, the detector, the sample compartment, the electronics, and the optics. So modified, the system can handle almost any luminescence measurement, at any temperature, in any atmosphere, in a magnetic field, and under experimental conditions dictated by the effect sought. A digitised detection system of photon counting is one of the Fluorolog features giving numerous advantages accrue from counting the actual photons impinging on the PMT cathode instead of measuring the resulting direct current, including a significantly improved signal to

noise ratio. Either a 150 or 450 W xenon arc can be plugged in. Normally operated cw, the lamp can be pulsed with a special phosphorimetry attachment; then the pulse rate can be varied up to 60 Hz and the sampling time from 1 μ s to 10 ms. To maintain focus on a sample regardless of the excitation wavelength, and to assure maximum collection of light from the lamp, a large, off-axis ellipsoidal mirror was selected. The Fluorolog has been designed so that it can operate with a computer controlling both wavelength scanning and data gathering. A system incorporating the necessary computer and software is the Fluorocomp. This system not only acts as a data collection device, but also makes decisions in real time to change such parameters as integration time, scaling, and signal-to-noise (total number of photon counts). Subroutines already developed or being written include: repeat scanning of emission or excitation spectra; storage and retrieval of data in a floppy disc mass storage system: The Ramalog 5M/6M Laser Raman Spectrometers are the latest Spex laser Raman systems available.

Circle No. 53 on Reader Enquiry Card

Oriel introduce a CO₂ laser spectrum analyser which is a unique grating spectroscopy simultaneously displaying all the lasing transitions of a CO₂ laser. It is calibrated both in wavelength and rotational line designation to permit easy identification of 140 possible laser transitions between 9.1 and 11.3 μ m. These transitions are visually displayed through the use of an ultraviolet excited thermal sensitive screen which darkens in the area struck by the IR laser beam. The screen has a response time of 0.25 s and permits the instrument to resolve all the CO₂ rotational lines. The model 16 series is light, portable and can easily be introduced into laboratory setups.

Circle No. 54 on Reader Enquiry Card

Pye Unicam Ltd present a wide variety of spectrometers and associated equipment. The PW 1600 simultaneous X-ray spectrometer is a new multi-channel X-ray spectrometer where the total time for analysis of low or high alloy steel is 20 s, inclusive of loading of the sample via the air lock and exclusive print-out of the results. High accuracy is obtained through an integrated computer with advanced software that enables the user to take full advantage of the recent achievements in the field of inter-element corrections. For this purpose tables of influence coefficients can be supplied with the instrument to suit the user's

requirements. In this way the calibration of such instruments to analyse samples with a wide range of compositions has become a matter taking only one or two days and 6 to 30 standards. In the past such wide range calibration programs have sometimes required several weeks of work and up to 200 standards. The instrument has a high stability as is required for routine operation with a minimum of recalibration time needed. A compensation has only to be made for long term drift and this is done once every 8 hs. The drift correction is made for each individual analysing channel by means of a multi-element monitor situated in the 10-position sample turret in the spectrometer. The correction is on operator's command and is computer controlled. A combination of curved and flat crystals together with a new ground cathode end window X-ray tube and advanced measuring electronics gives the PW 1600 extremely short analysis times with high spectral resolution. The PV8350 direct reading emission spectrometer provides rapid analyses at relatively low cost. The concentrations of up to 20 elements, including carbon, sulphur and phosphorus, can be determined simultaneously in materials such as steel, iron, non-ferrous alloys, etc. The instrument is designed to respond reliably to the needs of foundries and steel plants for continuous production control analyses. Outstanding analytical performance and stability are achieved



by a combination of advanced optical and electronic design. The high resolving power, blazed grating optics of one metre radius cover the wavelength range 1,770 Å to 4,100 Å in the first order at a reciprocal dispersion of 4.63 Å mm⁻¹. The instrument's 50 Hz monoalternance source unit generates a special type of discharge of improved reproducibility. Modular electronics and freedom to choose the most appropriate measurement lines in each case, enable a PV8350 spectrometer to be tailor-made to match individual analytical requirements. Running under automatic control of a number of preselected programmes, the system

is simple to operate, and its excitation stand accommodates samples of a wide range of dimensions. The electronics can provide an automatic printout of the measured intensity values by a digital printer. There are alternative electronics for teletype printout and adaptation to a computer controlled emission spectrometer with direct readout in concentration. Other modules which can be incorporated within the standard spectrometer unit include a source unit operating at 500 Hz and offering the ultimate in analysis speed. Infrared spectrometers are being used in every type of industrial and scientific environment. The vast majority of applications require a fast, robust instrument combining flexibility, high performance and simplicity of use. All of these qualities can be found in a newly developed Double Beam infrared Spectrophotometer, the SP1050. The SP1050 has a linear wavenumber scale of 600–4,000 cm⁻¹, nichrome strip source, double beam optics and grating monochromator. It has solid state electronic systems and an integral strip chart recorder. The reliability and sensitivity of the company's IR50 Infrared Detector is now well proven and this successful detector has been incorporated into the design of the SP1050.

Circle No. 55 on Reader Enquiry Card

Zeiss introduces the new DM 4 Double-Beam Spectrophotometer. The Zeiss DM 4 is the latest addition to the Zeiss line of spectrophotometers that meets practically all requirements in absorption spectrophotometry for the ultra violet, visible and near infrared regions. This makes it a very efficient instrument in any analytical laboratory, for research in the material and life sciences, as well as for routine analyses wherever accurate and reproducible results are required. The great advantage of the DM 4 is that it is equally convenient to operate for rapid routine concentration analysis and kinetics as it is when it's used as a scanning ratio recording instrument for qualitative determinations. An exceptionally large and readily accessible sample compartment contains a 6-position sample changer which can be automated. Among its many features are automatic lamp switch-over, fool-proof push-button operation, precision Zeiss grating monochromator, and unique wave-length scan. The DM 4 has a spectral range from 195 to 850 nm, an absorbance range from -0.3 to 2.5 A, a concentration range from 0 to 10,000. It requires no more than 0.3 ml sample volume. Digital and analog outputs are provided.

Circle No. 56 on Reader Enquiry Card

announcements

Appointments

Professor S. T. Butler, to Head of the Nuclear Science and Technology Branch in the Australian Atomic Energy Commission, in charge of the AAEC Research Establishment at Lucas Heights, near Sydney.

Dame Kathleen Ollerenshaw, DBE, to succeed His Royal Highness The Duke of Edinburgh, KG, KT, as President of the Institute of Mathematics and its Applications on 1 January 1978. She is currently a Vice-President.

Dr A. E. Stuart, to Professor of Pathology, University of Newcastle-upon-Tyne, from 1 January 1978, and also as Head of the Department of Pathology for a period of five years from 1 January 1978. Dr Stuart succeeds Professor A. G. Heppleston.

Awards

The Dr W. Elenbaas prize for 1977 has been awarded to the British chemist **A. H. McKeag**, in recognition of his work in the field of luminescent materials.

Two Technology Writers' Awards, sponsored by ITT Business Systems under the auspices of the Association of British Science Writers, will be awarded for work published between 1 January and 31 December 1977. One will be for work published in professional periodicals, trade and technical magazines, or national and regional newspapers, the other for material broadcast on radio or television. Both prizes will be £1,000 cash and £500 for travel or equipment. All entries are by submission (to J. Anstiss, MPR Ltd, 293 Gray's Inn Road, London WC1, UK), and it is hoped to announce the winners in February 1978.

Meetings

24–26 October, **ASTM Symposium on Erosion: Prevention and Useful Applications**, Vail, Colorado (L. E. Burgess, ASTM, 1916 Race Street, Philadelphia, Pennsylvania 19103).

28 November–1 December, **International Conference on Optimisation of Sodium-cooled Fast Reactors**, London (British Nuclear Energy Society, 1–7 Great George Street, London SW1, UK).

29–30 November, **International Conference on the Future of Natural Fibres**, Manchester (J. K. Jackson, Shirley Institute, Didsbury, Manchester, UK).

30 November–2 December, **Conference on New Developments in Automatic Testing**, Brighton (The Institution of Electrical Engineers, Savoy Place, London, UK).

Person to Person

Exchange 2-bedroom furnished house in La Jolla, California, for flat in London, July–September 1978 to September 1979. Contact M. Friedkin, University of California San Diego, M-001, La Jolla, California, 92093.

Exchange 3-bedroom furnished house in San Francisco near UC Medical Center for similar in or near Central London for one year from July 1978. Contact H. E. Varmus, 956 Ashbury Street, San Francisco, California 94117.

Observations welcome from recent work in the field of increasing crop yields using an electrostatic field. The last reference I have, after Professor Lemstrom (1904), and Sir Oliver Lodge, is J. E. Newman, who conducted trials near Pershore, England, and reported increases in wheat yields of up to 20%, and also potato yield increases in experiments in Dumfries. (See Standard Handbook for Electrical Engineers, 5th edn, McGraw Hill). The work ceased about 1917. Contact A. F. Stobart, Manor Farm, Claydon, Banbury, Oxford, UK.

There will be no charge for this service. Send items (not more than 60 words) to Marcus Dobbs at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

3 December, **Public Educational Forum, 'The Recombinant DNA Controversy: Public Policy at the Frontier of Knowledge'**, San Francisco (J. Stubbs, Chair, Department of Cell and Molecular Biology, San Francisco State University, 1600 Holloway Avenue, San Francisco, California 94132).

5–7 December, **International Symposium on Mechanisms of Oxidising Enzymes**, La Paz, Mexico (Professor R. N. Ondarza, Consejo Nacional de Ciencia y Tecnologia Insurgentes Sur 1677–10° Piso, Mexico 20, DF Mexico).
5–9 December, **1977 Fall Meeting of the American Geophysical Union**, San Francisco (Meetings, AGU, 1909 K Street, NW, Washington, DC 20006, USA).

5–9 December, **International Conference on Radiation Measurement**, Atlanta (J. H. Kane, Special Assistant for Conferences, Energy Research and Development Administration, Washington, DC 20542).

6–8 December, **Symposium on Applications of Electroanalytical Sensors**, London (Sira Institute Ltd, South Hill, Chislehurst, Kent, UK).

9 December, **21st Meeting of the Peptide and Protein Group of the Chemical and Biochemical Societies**, London (Group Secretary, The Dyson Perrins Laboratory, South Parks Road, Oxford, UK).

12–14 December, **International Conference on the Monitoring of Hazardous Gases in the Working Environment**, London (The Chemical Society, Burlington House, Piccadilly, London W1, UK).

12–15 December, **Conference on Conservation, Management and Development of Recreational Freshwater Fisheries**, Oxford (Water Research Centre, Medmenham Laboratory, Henley Road, Medmenham, PO Box 16, Marlow, Bucks, UK).

13–14 December, **Symposium on Electrocrystallisation, Nucleation and Phase Formation**, Southampton (Faraday Division, The Chemical Society, Burlington House, Piccadilly, London W1, UK).

14 December, **Ion Sputtering and Depth Profiling in Surface Analysis**, London (Institute of Physics, 47 Belgrave Square, London SW1, UK).

20–21 December, **Symposium of the Nucleotide Group, Chemical and Biochemical Societies, on Nucleoside and Polynucleotide Synthesis and Structure**, Birmingham, UK (Please send addressed envelope (stamped if from UK) to Dr R. T. Walker, Chemistry Department, Birmingham University, PO Box 363, Birmingham, UK).

20–22 December, **Cell Motility**, London (M. E. J. Holwill, Department of Physics, Queen Elizabeth College, London W8, UK).

Meetings 1978

3-7 January, **International Astronomical Union Colloquium on Protostars and Planets**, Tucson (T. Gehrels, Lunar and Planetary Laboratory, University of Arizona, Tucson, Arizona 85721).

4-5 January, **Fishfarming and Wastes**, London (Society of Chemical Industry, Water and Environment Group, 135 New London Road, Chelmsford, UK).

4-6 January, **15th Annual Solid State Physics Conference**, Coventry (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

24 January, **Acute and Chronic Effects of Radiation on Man**, London (Professor J. H. Martin, Department of Medical Biophysics, Blackness Laboratory, University of Dundee, Dundee, UK).

25-27 January, **Conference on Muscular Dystrophy and Other Inherited Diseases of Skeletal Muscle in Animals**, New York (Conference Department, The New York Academy of Sciences, 2 East 63rd Street, New York, NY 10021).

26 January, **Symposium on Internal Corrosion of Water Systems**, London (Department of Industry, Committee on Corrosion Secretariat, Room 540, Abell House, John Islip Street, London SW1, UK).

23-26 February, **Asian Congress of Parasitology**, Bombay (D. M. Renapurkar, Secretary General, Asian Congress of Parasitology, Haffkine Institute, Bombay, India).

27 February-3 March, **29th Annual Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy**, Cleveland (United States Steel Corporation Research Laboratory, MS 57, Monroeville, Pennsylvania 15146).

6-10 March, **International Colloquium on Earth Observation from Space and Management of Planetary Resources**, Toulouse, France (Secretariat du colloque, OST, BP No. 4130, 31030 Toulouse Cedex, France).

9-11 March, **International Symposium on Quantum Biology and Quantum Pharmacology**, Palm Coast, Florida (Director, Sanibel Symposia, Williamson Hall, University of Florida, Gainesville, Florida 32611).

29 March-1 April, **3rd International Conference on Submillimetre Waves and their Applications**, Guildford (Institute of Physics, 47 Belgrave Square, London SW1, UK).

2-6 April, **2nd Argentine Congress of Palaeontology and Biostratigraphy and the 1st Latinoamerican Congress of Palaeontology**, Buenos Aires (II Congreso Argentino de Paleontología y Biostratigrafía y I Congreso Latino-

americano de Paleontología, Maipú 645, Primer Piso, 1006-Buenos Aires, Republica Argentina).

10-12 April, **International Conference on Geological Information**, London (International Conference on Geological Information, c/o Palaeontology Library, British Museum (Natural History), Cromwell Road, London SW7, UK) (for those resident in N and S America: D. C. Ward, University Libraries, University of Colorado at Boulder, Boulder, Colorado 80309).

10-12 April, **Symposium on Microprocessors in Analytical Instrumentation**, London (Gerda Holt, Scientific Symposia Ltd, 42-43 Gerrard Street, London W1, UK).

10-13 April, **Utilisation of Sewage Sludge on Land**, Oxford (The Conference Organiser, Water Research Centre, Medmenham Laboratory, Henley Road, Medmenham, PO Box 16, Marlow, Bucks, UK).

11-12 April, **Symposium on the Use of Alternatives in the Discovery, Development and Testing of Therapeutic Products**, London (Symposium Organiser, FRAME, 312a Worple Road, Wimbledon, London SW20, UK).

11-13 April, **Colloid Stability**, Lunteren, The Netherlands (Faraday Division, The Chemical Society, Burlington House, London W1, UK).

Reports & Publications

Other countries—August

Energy, Mines and Resources Canada. Geological Survey of Canada. Bulletin 262: The Origin and Migration of Petroleum in the Western Canadian Sedimentary Basin, Alberta—a Geochemical and Thermal Maturation Study. By G. Deroot, T. G. Powell, B. Tissot and R. G. McCrossan. With contributions by P. A. Hacquebard. Pp. xvii + 136. \$12. Bulletin 271: The Use of Pedological Studies in Interpreting the Quaternary History of Central Yukon Territory. By A. E. Foscolos, N. W. Rutter and O. L. Hughes. Pp. 48. \$6. Bulletin 275: Early Paleozoic Ostracoda from Southwestern District of Mackenzie and Yukon Territory. By M. J. Copeland. Pp. 88 (16 plates). (Ottawa: Geological Survey of Canada, 1977.) [48]

Australia: Commonwealth Scientific and Industrial Research Organization. Division of Environmental Mechanics—Report for 1975-76. Pp. 37. (Canberra City: CSIRO, 1977.) [59]

Cognitive Therapy and Research. Vol. 1, No. 1, March 1977. Edited by Michael J. Mahoney. Published quarterly. Pp. 1-98. Subscription rates: Vol. 1, 1977 (4 issues) \$36; Individual subscribers \$18. (New York: Plenum Publishing Corporation, 1977.) [59]

CERN—European Organization for Nuclear Research. CERN 77-12: The SPS Auxiliary Magnet Power Supplies. By H. C. Appelo and S. van der Meer. Pp. v + 23. (Geneva: CERN, 1977.) [59]

France. Commissariat à l'énergie Atomique. Rapport En Annuel 1976. Pp. 110. Activités Scientifiques et Techniques 1976. Pp. 238. (Paris: Commissariat à l'énergie Atomique, 31-33 Rue de la Fédération, 1977.) [88]

Institut für die Pädagogik der Naturwissenschaften/Institute for Science Education (West Germany). The IPN—Its Structure and Functions. (IPN Report-Inf-Brief). Pp. 82. IPN-Materialien, Übersicht 76. Pp. 187. (Kiel: Institute for Science Education (FR Germany), Olshausenstrasse 40-60, 1976.) [88]

US Department of Health, Education and Welfare. Public Health Service, National Institutes of Health. Public Accountability and Peer Review in Health Care Delivery in the United States and the United Kingdom. By S. Palmer. Pp. vi + 31. (Washington, DC: US Government Printing Office, 1977.) [88]

Rheinisch-Westfälische Akademie der Wissenschaften. Vorträge N. 169: Cytoplasmatische Actomyosine und ihre Bedeutung für Zellbewegungen. Von Karl Ernst Wihlfarth-Boettmann. Anaerobier Stoffwechsel bei Wirbellosen Tieren. Von Ernsy Zebe. Pp. 77. (Wiesbaden: Westdeutscher Verlag, 1977.) DM 80. [88]

United States Department of the Interior: Geological Survey. Professional Paper 813-G: Summary Appraisals of the Nation's Ground-Water Resources—Great Basin Region. By Thomas E. Eakin, Don Price and J. R. Harrill. Pp. iv + 37 + plate 1. (Washington, DC: US Government Printing Office, 1976.) [88]

Institute of African Studies: Africa Documentation Center. Africa Documentation Service. Series A, No. 14: Drought in Africa: Climatic, Ecological, Socio-Economic Aspects. By Marianne Weiss and Anne Jansen. Pp. 115. (Hamburg: Institut für Afrika-Kunde, Dokumentations-Leitstelle, Neuer Jungfernstieg, 21, 1976.) DM 10. [98]

Center for Disease Control: Abortion Surveillance, 1975. Pp. iv + 49. (Atlanta, Georgia: US Department of Health, Education and Welfare, Public Health Service, Center for Disease Control, Bureau of Epidemiology, Family Planning Evaluation Division, 1977.) [98]

US Department of Health, Education and Welfare. Development and Evaluation of a Hexokinase/Glucose-6-Phosphate Dehydrogenase Procedure for Use as a National Glucose Reference Method. By Jane W. Neese, Patricia Duncan, David Bayse, Mary Robinson, Teresa Cooper and Charles Stewart. Pp. vi + 147. (Atlanta, Georgia: US Department of Health, Education and Welfare, Public Health Service, Center for Disease Control, Bureau of Laboratories, Clinical Chemistry Division, 1976.) [118]

Adi Sankara Advaita Research Centre. Research Bulletin No. 1: Reflections and Speculations on Space, Time and Causality. By Dr T. S. Shankara. Pp. iv + 12. (Madras: Adi Sankara Advaita Research Centre, 28 A.N.H. Road, 1977.) Rs. 3; \$2. [118]

United States Department of the Interior: Geological Survey. Water-Supply Paper 2162: Ground-Water Levels in the United States, 1971-74. Southwestern States. Pp. v + 86. (Arlington, Va.: Branch of Distribution, US Geological Survey, 1200 South Eads Street, 1977.) [128]

National Research Council Canada. Associate Committee on Scientific Criteria for Environmental Quality. NRCC No. 15389: Fentitrothion—The Long-Term Effects of Its Use in Forest Ecosystems, Current Status. Pp. iv + 18. (Ottawa: National Research Council Canada, 1977.) [158]

United States Department of the Interior: Geological Survey. Water-Supply Paper 2163: Ground-Water Levels in the United States, 1972-74. North-Central States. Pp. v + 89. (Washington, DC: US Government Printing Office, 1977. For sale by the Branch of Distribution, US Geological Survey, 1200 South Eads Street, Arlington, VA.) [158]

Food and Agriculture Organization of the United Nations. Report on the Ad Hoc Consultation on a Scheme for Agricultural Credit Development, Rome, 3-4 June 1976. Pp. 24. 97p. FAO Fisheries Technical Paper No. 159: Production, Trade and Utilization of Seaweeds and Seaweed Products. By J. Naylor. Pp. vi + 73. £2.60. (Rome: FAO, London: HMSO, 1976.) [158]

National Research Council Canada. Associate Committee on Scientific Criteria for Environmental Quality. NRCC No. 15387: Medicare Data—Its Use in Defining the Effects of the Environment on Health. By C. A. R. Dennis, R. Drope and D. Matz. Pp. 164. (Ottawa: Publications, NRCC, 1977.) \$2.50. [178]

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Computing Research, 1975/1976. Pp. iii + 71. Division of Chemical Technology: Research Review 1976. Pp. 151. (Melbourne: CSIRO, 1977.) [188]

United States Department of the Interior: Geological Survey. Water-Supply Paper 1887: Maximum Floodflows in the Conterminous United States. By J. R. Crippen and Conrad D. Bue. Pp. iv + 52. (Washington: US Government Printing Office, 1977.) [198]

Council of Europe. European Information Centre for Nature Conservation. Selective Bibliography on Wetlands. (Documentation Series, No. 2.) Pp. iii + 38. (Strasbourg: Council of Europe, 1977.) [198]

US Department of Health, Education and Welfare. Public Health Service. Alcohol, Drug Abuse, and Mental Health Administration. Drugs and Driving. Edited by Robert E. Willette. (NIDA Research Monograph, 11.) Pp. xii + 137. (Washington, DC: US Government Printing Office, 1977.) \$1.70. [228]

International Atomic Energy Agency. The Annual Report for 1976. Pp. 62. The Agency's Budget for 1978. Pp. v + 119. (Vienna: International Atomic Energy Agency, 1977.) [228]

Regional Research Laboratory, Hyderabad. Annual Report, 1975/1976. Pp. xvii + 157. (Hyderabad: Regional Research Laboratory, 1977.) [238]

Hoechst Aktiengesellschaft. Annual Report 1976. Pp. 69. (Frankfurt am Main: Hoechst Aktiengesellschaft, 1977.) [248]

Collections of Research Papers Published in Foreign Countries Selected from Grants of NSC, Republic of China, 1972-1975. Vol. 3: Mathematics. Pp. 317-604. Vol. 4: Physics. Pp. vi + 605-1152. Vol. 5: Agriculture. Pp. iii + 1153-1335. Vol. 8: Medicine (2). Pp. vii + 1985-2581. Vol. 9: Engineering and Applied Sciences. Pp. vi + 2583-2973 + 57. (Taipei, Taiwan: National Science Council, 2 Canton Street, 1976.) [248]

United States Department of the Interior: Geological Survey. Bulletin 1433: Eocene Rocks in Northeast Washington—Radiometric Ages and Correlation. By R. C. Pearson and J. D. Obradovich. Pp. iv + 41 + plate 1. (Washington, DC: US Government Printing Office, 1977.) [258]

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The Bushmen. Pp. 38. (Cape Town: Trustees of the South African Museum, 1976.) [268]

Electric Power Systems Research. Vol. 1, No. 1, September 1977. Pp. 1-1-86. 1977 subscription: volume 1, 4 issues. Institutional subscription: SFrs. 145; \$60. Personal subscription: SFrs. 50; \$20. (Lausanne: Elsevier Sequoia, S.A., 1977.) [268]